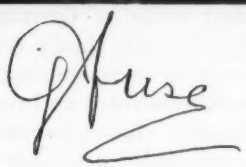
The Tohoku Journal of Experimental Medicine, Vol. 63, Nos. 2-3, 1956

Gennosuke Fuse. In Memoriam



We regret to record the death on December 12th, 1946, of Professor Gennosuke Fuse, who for twenty-six years until his retirement at the age limit held the chair of Anatomy at Tohoku University.

Professor Fuse was born in 1880, and after leaving Kanazawa High School in 1901, joined the Medical Faculty of Tokyo Imperial University.

He spent a few years in the anatomical laboratory of Y. Koganei in that university, then from 1907 to 1911 studied in Zürich, Switzerland, in the laboratory of C. v. Monakow. There he published several good papers, and returned to Japan to serve as a professor of anatomy in the newly established Niigata Med-

ical School. In 1914 he worked again with Monakow to write "Mikroskopischen Atlas des menschlichen Hirns, I. Medulla oblongata".

In 1915 Fuse was appointed professor of anatomy in the Imperial University of Sendai, and directed the arrangement and administration of the newly constructed laboratory there. As the chief of the laboratory, he took charge in fact of two chairs of anatomy, and among the many young anatomist pupils who studied under his charge were many who later came to be well known: Kodama in Sapporo, Seto and Yamazaki in Sendai, Ogawa and Yamada in Tokyo, Katsumata in Yokohama, Suzuki in Hiroshima, Fukuyama in Fukushima, and late Ikeda in Nagasaki.

From his laboratory in 1919, Fuse published "Arbeiten aus dem anatomischen Institut der Kaiserlich-Japanischen Universität zu Sendai", and continued the issue until 1942, ending with No. 25. In the main the publication was made up of the papers from his laboratory, many of which were his papers. Of the total 145 essays and 5261 pages, he contributed 40 papers and 1992 pages. Besides these essays he published 16 papers including 630 pages. The list of all his works is inserted in the next pages. He was a very accomplished writer of German.

Briefly stated, Fuse's work chiefly comprises microscopical studies on the brain stem of various mammals, including human beings, and in 1920 he was awarded an Imperial Prize by the Imperial Academy of Science. Later, in 1946, in appreciation of his good work, he was nominated a member of the Academy.

Fuse had interest in students activities and by his good offices medical students could have their dormitory, by the benevolence of a business man. Student houses and school-grounds were much developed and improved while he was a dean, in 1925–1927. A scholar, a students' friend, and a revered tutor passed away when he died.

Y. SATAKE
Editor

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1911 to 1941

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Histochemical Study on the Liver of Patients with Cancer

By

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With the recent progress of cytochemistry attention has been paid to phosphatases, which takes an important role in the metabolism of energy-rich phosphates in living organisms, and the relationship between this group of enzymes and malignant tumors has become clearer and clearer. Thus, Mulay and Firminger[1] have found that phosphatase activity in the serum and liver of rats bearing tumors is higher than that of normal rats; and Bernhard and Rosenbloom[2] have reported that the serum phosphatase of patients with cancer gives higher values than normal.

As to glycogen, which occupies a principal position in the problem of carbohydrate metabolism there have been published numerous reports since Meixner.[3] Literature of studies with liver pieces from living human beings, however, is very scanty.

We have, therefore undertaken an investigation in which small specimens were removed from the liver of patients with surgical diseases in the upper abdomen immediately on laparotomy, and were investigated histochemically in regard to their phosphatases and glycogen. When the cases were classified into two groups, cancer and non-cancer, the results led to interesting conclusions as will be reported in the following.

I. Alkaline phosphatase

Material and method.

The cases investigated were 29 cancer cases (26 cancer cases of the stomach, 2 of the gallbladder, 1 of the pancreas) and 14 non-cancer cases (7 cases of gastric or duodenal ulcer, 1 of gastroptosis, 2 of cholelithiasis, 1 of cholecystitis, 1 of pancreatic cyst, 2 of hepatic cirrhosis accompanying congestive splenomegaly).

A small specimen was removed from the liver immediately on laparotomy, soon fixed in acetone, incubated for 24 hours in a substrate of pH 9.4 by a modification of Gomori's method[4], and examined microscopically.

TABLE I

Alkaline Phosphatase Activity of the Liver

	Area	Liver tuble								Cytoplasm				Cell membrane				Bile capillary				Sinusoid			
		Central				Peripheral																			
	Activity	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)
Cancer of	Stomach 26 cases	13	9	4	0	11	8	7	0	10	11	5	0	9	12	5	0	10	8	8	0	15	7	4	0
	Gallbladder 2 »	2	0	0	0	2	0	0	0	1	1	0	0	1	1	0	0	0	2	0	0	2	0	0	0
	Pancrease 1 case	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0
	Total 29 cases	16	9	4	0	14	8	7	0	12	12	5	0	11	13	5	0	10	11	8	0	18	7	4	0
	Percentage	55	28	14	0	48	28	24	0	41	41	17	0	38	45	17	0	34	38	28	0	62	24	14	0
Non-cancer	Gastric or duodenal ulcer 7 cases	1	3	3	0	1	1	5	0	1	1	5	0	1	1	5	0	1	1	4	1	0	2	5	0
	Gastroptosis 1 case	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
	Cholelithiasis 2 cases	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	1	1	0	0	1	1	0	0
	Pancreatic cyst 1 case	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0
	Hepatic cirrhosis 2 cases	0	1	1	0	0	1	1	0	0	0	2	0	0	0	2	0	0	0	2	0	0	2	0	0
	Cholecystitis 1 case	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0
	Total 14 cases	4	5	5	0	4	3	7	0	4	2	8	0	4	2	8	0	2	4	7	1	2	6	6	0
	Percentage	29	36	36	0	29	21	50	0	29	14	57	0	29	14	57	0	14	29	50	7	14	43	43	0

Nuclearmembrane				Karyolymph				Nucleolus				Chromatin				Capsule of Glisson											
																Bile duct				Vein				Artery			
High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)
12	10	4	0	10	10	6	0	17	8	1	0	13	11	2	0	0	0	14	12	17	6	3	0	0	0	19	7
1	1	0	0	1	1	0	0	2	0	0	0	1	1	0	0	0	0	2	0	1	1	0	0	0	0	2	0
1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0
14	11	4	0	12	11	6	0	20	8	1	0	15	12	2	0	0	0	17	2	19	7	3	0	0	0	22	7
48	38	14	0	41	38	21	0	69	28	3	0	52	41	7	0	0	0	59	41	66	24	10	0	0	0	76	24
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3	3	8	0	3	4	7	0	4	6	4	0	2	3	9	0	0	2	6	6	4	7	3	0	0	1	7	6
21	21	57	0	21	29	50	0	29	43	29	0	14	21	64	0	0	14	43	43	29	50	21	0	0	7	50	43

Fig. 1

Fig. 2

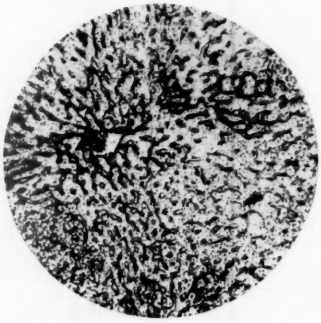


Fig. 1. Alkaline phosphatase activity of a liver tubule in a case of gastric cancer. The central vein and sinusoids show a high activity; liver cells and their nuclei, a medium activity.

Fig. 2. Alkaline phosphatase activity of a liver tubule in a case of gastric cancer. Sinusoids in the central area show a high activity and some parts in the periphery show fatty degeneration.

Fig. 3

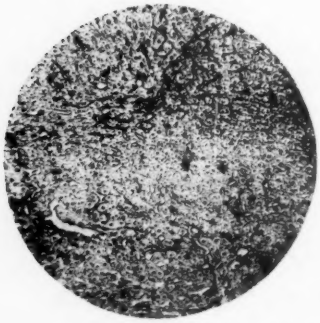


Fig. 4

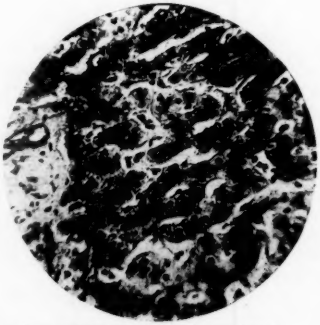


Fig. 3. Alkaline phosphatase activity of a liver tuble in a case of duodenal ulcer. Sinusoids in the central and peripheral areas of the tubule show a medium activity; the intermediate area and bile capillaries, a low activity.

Fig. 4. Alkaline phosphatase activity of a liver tubule in a case of cholelithiasis. The expansion of sinusoids, the narrow of liver cell cords (Staung's picture), and bile capillaries show a high activity; also veins and small arteries in Glisson's capsule are high in activity.

The results were read as high, medium, low, and none (−) according to the height of liver alkaline phosphatase activity, by referring to Sulkin and Gardner's[5] method.

Experimental results (Tables I, Figs. 1–5)

First, the phosphatase activity of a liver lobule as a whole was higher

Fig. 5

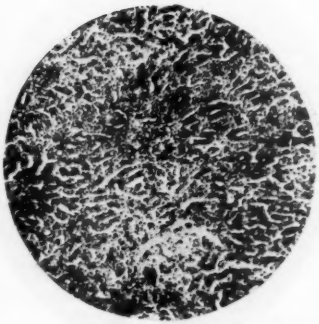


Fig. 6

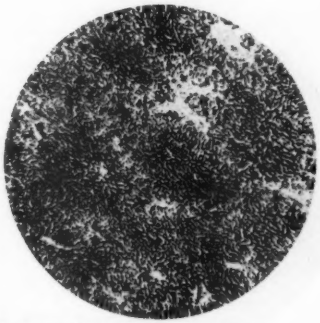


Fig. 5. Alkaline phosphatase activity of a liver tubule in a case of hemorrhagic gastric ulcer. The expansion of sinusoids ; bile capillaries, and sinusoids show a high activity ; also liver cells and nuclei are high in activity.

Fig. 6. Acid phosphatase activity of a liver tubule in a case of gastric cancer. Bile capillaries, liver cells, and nuclei especially in the central area of the tubule show a high activity.

Fig. 7

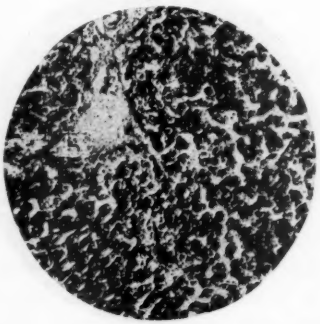


Fig. 8

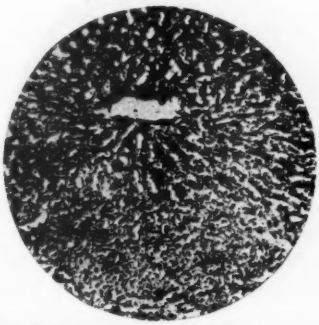


Fig. 7. Acid phosphatase activity of a liver tubule in a case of cholelithiasis. Liver cells and nuclei show a high activity ; in sinusoids, only the nuclei of astral cells show some activity.

Fig. 8. Acid phosphatase activity of a liver tubule in a case of gastric cancer. Liver cell nuclei show a high activity ; Sinusoids show a medium activity.

in cancer cases than in non-cancer cases, which was true both in the central and in the peripheral areas.

As for the phosphatase activity of different parts of a liver lobule, it was found that the activity of liver cells, bile capillaries, and sinusoids was higher in cancer cases than in non-cancer cases, and especially sinusoids and bile capillaries showed marked differences in the activity between

Fig. 9

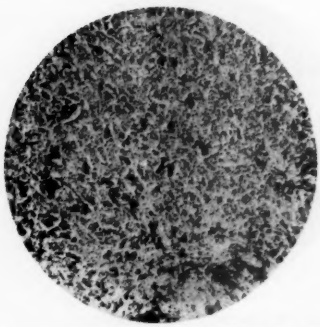


Fig. 10

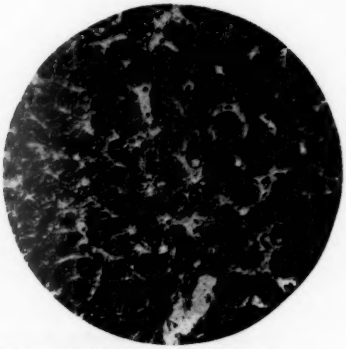


Fig. 9. Acid phosphatase activity of a liver tubule in a case of hepatic cirrhosis. Bile pigment is seen in the liver cell cords; no activity.

Fig. 10. Glycogen in a liver tubule in a case of cholelithiasis. Glycogen content, ###; distribution, uniform and diffuse.

Fig. 11

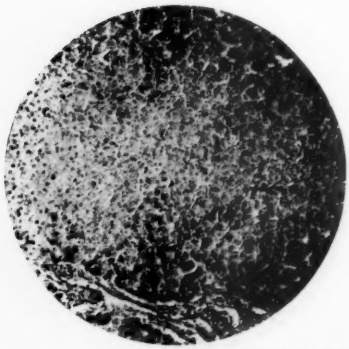


Fig. 12

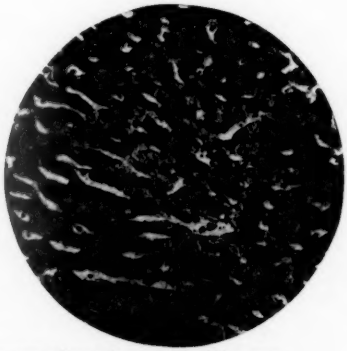


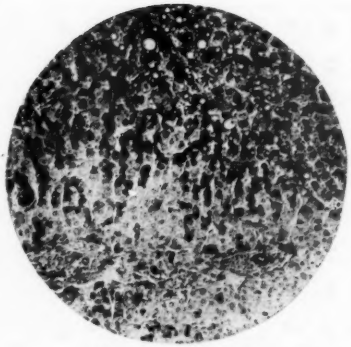
Fig. 11. Glycogen in a liver tubule in a case of gastric cancer. Glycogen content, ##; distribution, central and peripheral.

Fig. 12. Glycogen in a liver tubule in a case of hemorrhagic gastric ulcer. Glycogen content, ##; distribution, mosaic.

cancer and non-cancer cases. Inside the nuclues of a liver cell, the nucleolus and chromatin in particular showed a high activity in cancer cases.

In Glisson's capsule, only the veins in cancer cases represented a higher activity, the bile ducts and arteries showing no high activity.

Thus, it is to be noted that the alkaline phosphatase activity of the liver in cancer cases was much higher than in non-cancer cases; this was especially remarkable in sinusoids, liver cell nucleoli, and in the veins of Glisson's capsule. These differences in the activity between both cases



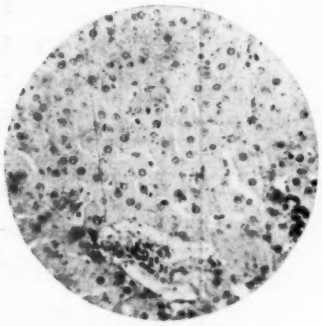


Fig. 13. Glycogen in a liver tubule in a case of cancer of the gallbladder. Glycogen content, ++ ; distribution, central ; the central area shows fatty degeneration.

Fig. 14. Glycogen in a liver tubule in a case of cancer of the pancreas. Glycogen content, ± ; distribution irregular-insular.

were very conspicuous when only gastric or duodenal ulcer cases were compared with gastric cancer cases, with the exception of a case of hemorrhagic gastric ulcer in which a relief operation was performed for severe anemia. Further it will be seen that 2 cases of cholelithiasis and 1 case of cholecystitis also represented high activity, a fact which is in agreement with what has been reported by Wachstein, Rothman and others.[6-8]

II. Acid phosphatase

Material and method

The cases tested consisted of 26 cancer cases (23 cases of gastric cancer, 2 of cholecystitis, 1 of pancreatic cancer) and 14 non-cancer cases 6 cases of gastric or duodenal ulcer, 1 of gastroptosis, 3 of cholelithiasis, 1 of pancreatic cyst, 2 of hepatic cirrhosis accompanying congestive splenomegaly, 1 of cholecystitis).

A small specimen was removed from the liver immediately on laparotomy, soon fixed in acetone, incubated for 2 to 24 hours in a substrate of pH 4.5 by Takeuchi's blood phosphatase method[9], and examined microscopically. The results were read in the same manner as high, medium, low, (incubated for 4 hours) and none (—) (incubated for 24 hours).

Experimental results (Tables II, Figs. 6–9)

First, a liver tubule as a whole showed higher activity of acid phosphatase in cancer cases than in non-cancer cases as before, though no activity was observed in not a few cases of both groups.

The results obtained with different parts of a liver tubule, such as

TABLE II

Acid Phosphatase Activity of the Liver

	Area	Liver tubule								Cytoplasm				Cell membrane				Bile capillary				Sinusoid			
		Central				Peripheral																			
	Activity	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)
Cancer of	Stomach 23 cases	12	0	3	8	12	0	3	8	10	2	3	8	0	2	13	8	5	1	6	11	0	3	10	10
	Gallbladder 2 „	1	1	0	0	2	0	0	0	1	1	0	0	0	0	2	0	0	0	2	0	0	1	0	0
	Pancreas 1 case	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
	Total 26 cases	14	1	3	8	15	0	3	8	12	3	3	8	0	2	16	8	5	1	9	11	0	5	11	10
	Percentage	54	4	12	31	58	0	12	31	46	12	12	31	0	8	62	31	19	4	35	42	0	19	42	38
Non-cancer	Gastric or duodenal ulcer 6 cases	1	1	1	3	1	1	1	3	1	1	1	3	0	0	3	3	0	0	2	4	0	0	3	3
	Gastroptosis 1 case	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
	Cholelithiasis 3 cases	2	1	0	0	2	1	0	0	1	2	0	0	0	0	3	0	0	0	2	1	0	0	3	0
	Pancreatic cyst 1 case	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0
	Hepatic cirrhosis 2 cases	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2
	Cholecystitis 1 case	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0
	Total 14 cases	4	2	2	6	4	2	2	6	3	3	2	6	0	0	8	6	1	0	4	9	0	0	8	6
	Percentage	29	14	14	43	29	14	14	43	21	21	14	43	0	0	57	43	7	0	29	64	0	0	57	43

Nuclear-membrane				Karyolymph				Nucleolus				Chromatin				Capsule of Glisson											
																Bile duct				Vein				Artery			
High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)	High	Medium	Low	(−)
11	4	0	8	10	4	1	8	12	3	0	8	11	4	0	8	0	0	3	20	0	0	1	22	0	0	0	23
1	1	0	0	1	1	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	1	0	0	1	1
1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
13	5	0	8	12	5	1	8	14	3	1	8	13	4	1	8	0	0	4	22	0	0	3	23	0	0	1	25
50	19	0	31	46	19	4	31	54	12	4	31	50	15	4	31	0	0	15	85	0	0	12	88	0	0	4	96
2	1	0	3	2	1	0	3	2	1	0	3	2	1	0	3	0	0	1	5	0	0	2	4	0	0	0	6
0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
3	0	0	0	3	0	0	0	3	0	0	0	3	0	0	0	1	1	1	0	0	0	2	1	0	0	1	2
1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1
0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2
1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
7	1	0	6	7	1	0	6	7	1	0	6	7	1	0	6	1	1	3	9	0	0	5	9	0	0	1	13
50	7	0	43	50	7	0	43	50	7	0	43	50	7	0	43	7	7	21	64	0	0	36	64	0	0	7	93

liver cells, bile capillaries, and sinusoids, are given in Table II, where it will be seen that the activity was especially high in the cytoplasm and bile capillaries of cancer cases, while sinusoids and the cell membrane showed no high activity even in cancer cases, a result which is different from that with alkaline phosphatase. No differences in the activity of liver cell nuclei were found between both groups of cases, nearly half the cases showing a high activity in each group.

The results with the bile ducts, veins, and arteries in Glisson's capsule are shown in Table II, where it will be seen that with the exception of fairly high activity of the bile duct in non-cancer cases no definite differences in their activity were noticed between both groups of cases, the majority of the cases in each group being negative in the activity.

Thus, it is to be noted that generally the acid phosphatase activity of the liver also was higher in cancer cases than in non-cancer cases though not to an extent so remarkable as the alkaline phosphatase activity (fairly large differences being found only in the cytoplasm and bile capillaries). In addition, the cases of hemorrhagic gastric ulcer, cholelithiasis, and cholecystitis showed a high activity like alkaline phosphatase activity.

III. Glycogen

Material and method

The cases tested consisted 33 cancer cases (29 cases of gastric cancer, 3 of cancer of the gallbladder and biliary tract, 1 of pancreatic cancer) and 14 non-cancer cases (6 cases of gastric or duodenal ulcer, 1 of gastroptosis, 3 of cholelithiasis, 1 of pancreatic cyst, 2 of hepatic cirrhosis accompanying congestive splenomegaly, 1 of cholecystitis).

A small specimen was removed from the liver immediately on laparotomy, soon fixed in Carnoy's fluid, stained by Hotchkiss's[10] and McManus's[11] methods, and examined microscopically. The results were read as follows: ###, all liver cells containing much glycogen; ##, nearly all cells containing glycogen; #, glycogen content being smaller than ## but larger than +; +', glycogen content being small; ±, traces of glycogen being demonstrated in one to several cells; −, glycogen being completely negative. The distribution of glycogen among various parts of liver tubules was also examined.

Experimental results (Table III, Figs. 10–14)

The results are summarized in Table III. First, it will be seen that those cancer cases with a large glycogen content (###, ##) were less in number than those non-cancer cases. If a comparison is made between the cases of gastric cancer and the cases of gastric or duodenal ulcer or between the cases of cancer of the gallbladder and biliary tract and the cases of cholelithiasis, these differences will be more remarkable. The ± case

was found only in a single case of pancreatic cancer.

As for the distribution of glycogen, those cases in which the distribution was uniform and diffuse or localized to the central area were nearly equally found in both groups of cases, not a single case of peripheral distribution being observed; these findings support what has been maintained by Arndt[12] and others. Irregular-insular distribution was noticed in only 2 cases of cancer; and mosaic distribution was encountered in each 1 case of gastric cancer, gastric ulcer, and gastroptosis. It must be taken into account, however, that considering the nature of glycogen, about 24

TABLE III

Glycogen Content and Distribution in a Liver Tubule

	Case \ Glycogen	Content						Distribution						
		###	##	#	+	±	−	Uniform & diffuse	Central	Central & peripheral	Peripheral	Subcapsular	Irregular-insular	None
Cancer of	Stomach 29 cases	7 | 9 / 55%	8	5	0	0		14	10	4	0	0	1	0
	Gallbladder and biliary tract 3 cases	1 | 0 / 33%	1	1	0	0		2	1	0	0	0	0	0
	Pancreas 1 case	0 | 0 / 0%	0	0	1	0		0	0	0	0	0	1	0
	Total 33 cases	8 | 9 / 52%	9	6	1	0		16	11	4	0	2	0	0
Non-cancer	Gastric or duodenal ulcer 6 cases	1 | 4 / 83%	1	0	0	0		4	2	0	0	0	0	0
	Gastroptosis 1 case	0 | 1 / 100%	0	0	0	0		1	0	0	0	0	0	0
	Cholelithiasis 3 cases	1 | 2 / 100%	0	0	0	0		1	2	0	0	0	0	0
	Pancreatic cyst 1 case	0 | 0 / 0%	1	0	0	0		0	1	0	0	0	0	0
	Hepatic cirrhosis 2 cases	0 | 0 / 0%	2	0	0	0		0	2	0	0	0	0	0
	Cholecystitis 1 case	0 | 0 / 0%	0	1	0	0		0	0	1	0	0	0	0
	Total 14 cases	2 | 7 / 64%	4	1	0	0		6	7	1	0	0	0	0

hours' fasting before operation and the amounts of preoperative transfusion of blood and other fluids might necessarily affected glycogen distribution. And indeed, all the cases dealt with in this examination received in the period of 1 to 3 days before operation 1000 cc. of 5 per cent glucose, 100 to 300 cc. of 20 per cent glucose, 100 to 1000 cc. of blood, 1000 cc. of physiological saline, and 100 to 500 cc. of blood plasma, the exact amounts of fluids given being not always the same in all cases. Thus, I cannot go too

far in discussing the results in regard to glycogen, but it may well be said at least that liver glycogen content of patients with cancer at the time of laparotomy was smaller than that of non-cancer patients in many cases, being suggestive of some functional diturbances of liver cells especially in their ability of forming glycogen.

Summary and Conclusions

A small specimen was removed from the liver at the time of laparotomy for various surgical operations and was submitted mainly to histochemical investigations, with the results to be summarized as follows:

1. In patients of cancer the liver activity in acid and alkaline phosphatases was higher than in non-cancer patients. High phosphatase activity was often encountered even in those cases which had not been found abnormal by the examination of liver function.

2. The glycogen content of the liver of patients with cancer was much lower than that of non-cancer patients. In those cases which had been found more or less abnormal in liver function the glycogen content was always lower than ##, being suggestive of a parallelism between liver function and glycogen content.

3. Considering these results concerning phosphatase activity and glycogen of the liver, at the time of laparotomy the liver of patients with cancer was possibly under worse conditions than the liver of non-cancer patients, and it is to be stressed that certain treatments are necessary for protection of the liver of patients with cancer.

In conclusion, I wish to express my hearty thanks to Assistant Professor Tomu Mori of the Anatomical Institute of the university for his kind guidance in histochemical staining techniques.

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Chemical Studies of Carcinoma

XIV. A Toxic Substance Found in Gastric Carcinoma and its Effect on the Excised Frog Heart

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It is generally accepted that a cancerous tissue contains some toxic substances, but the nature of these substances has not been elucidated, opinion on this matter being divided[1)2)]. Recently, Greenfield and Meister[3)] and Nakahara[4)] have reported the existence of a toxohormone in the dialyzate of the aqueous extract of a tumor tissue based on the results that the dialyzate lowered the catalase activity of the liver.

With a view of clarifying the nature of toxic substances contained in cancer, as a part of a series of studies being made by Assistant Professor K. Oh-Uti and his coworkers in our clinic, I investigated the effect of extracts of gastric carcinoma on the excised frog heart, and recognized their remarkable inhibiting action, which was not shown by the corresponding extracts of normal human gastric mucosa. By a fractionation study this inhibition has been traced to a substance in the portion of the extract which passed through the cellophane membrane on dialysis. So far as is known, the work to be reported here is the first one dealing with the inhibiting action of the gastric carcinoma extract on the frog heart.

Experimental

Tests of the Gastric Carcinoma Extract

The frog used was Rana nigromaculata, weighing 20 to 50 g. The effect of an extract on an excised frog heart was recorded kymographically by the Shioya's third method at room temperature (17 to 27°C).

The extracts were prepared as follows: A sample of gastric carcinoma or normal gastric mucosa was taken from a specimen of gastric carcinoma or of gastric and duodenal ulcer excised in our clinic, minced, and washed well in water to remove blood constitutens and necrotic tissue. After removing moisture with filter paper as far as possible, 90 cc. of Ringer's solution for frogs (hereafter to be referred to simply as "Ringer's") or

distilled water was added to 10 g. of a tissue, and the mixture was kept at room temperature for 24 hours with constant shaking to facilitate the extraction, and then centrifuged. The supernatant thus obtained was used as the extract of that tissue. A fresh sample of gastric carcinoma or normal gastric mucosa was dried in vacuum to a mummy mass, to which was added distilled water or 2.5 per cent saline at the rate of 90 cc. per 10 g. of the dried material. The mixture was then treated in a similar manner to obtain the extract. The concentration of each the extract was so adjusted that the extract contained 1 g. of dry matter per 10 cc. by diluting the initial extract to a given extent estimated from the following previous test: A sample was taken from each extract, concentrated to a state of soup, and dried in vacuum to powder, which was weighed to determine the concentration of the extract. In experiments, a 100-fold dilution and its doubling dilutions were used.

1. *Effect of the extract of a fresh tissue in Ringer's solution for frogs*

The kymographic records obtained with varying dilutions of the extract of fresh gastric carcinoma in Ringer's are shown in Figure 1. At

Fig. 1. Effect of the extract of gastric carcinoma in Ringer's.
a. 1 :200 b. 1 :3200

a dilution of 1 :200 it will be seen that the inhibition was already remarkable immediately after exposure to the experimental solution, attained to its maximum after 12 seconds, then gradually decreased and almost disappeared after 42 seconds, a slight stimulatory effect being presented thereafter. The inhibition was perceptible also at dilutions up to 1 :800, very weak at 1 :1600, and not perceptible at 1 :3200.

In the case of normal gastric mucosa (Fig. 2) the effect was very slightly inhibitory at 1 :200 and 1 :400, and rather stimulatory at 1 :800.

Fig. 2. Effect of the extract of normal gastric mucosa in Ringer's.
a. 1 :200 b. 1 :800

2. *Effect of the extract of a fresh tissue in distilled water*

Tests of the aqueous extract of fresh gastric carcinoma or normal gastric mucosa were performed with doubling dilutions (diluted with Ringer's) of a 100-fold dilution of the extract, in the same manner as before. In the cases of both the extract of gastric carcinoma and that of normal gastric mucosa, the effect was remarkably inhibitory at 1 :100 and 1 :200; though the recovery of the heart began after 30 to 40 minutes, it was quite irregular, different from that in the case of an extract in Ringer's. As shown in Table I, the inhibition decreased with the increase of dilution

TABLE I

Effect of the Extracts Mummy-dried Materials in Distilled Water*

1:	100	200	400	800	1600
Gastric carc.	##	##	#	+	–
Normal gastric mucosa	##	#	–	–	–

##, D=0–15% ; #, 16–50%† +, 31–95% ; ±, higher than 96%. D (reciprocal of degree of inhibition)=

$$\frac{\text{Minimum amplitude of heat beat}}{\text{Normal amplitude}}$$

in both extracts, but more rapidly in the case of normal gastric mucosa. The dilution at which the inhibition first became negative was 1 :400 with normal gastric mucosa, and as high as 1 :1600 with gastric carcinoma.

3. *Effect of the extract of a dried tissue in distilled water*

The results obtained with the aqueous extracts of dried gastric carcinoma and normal gastric mucosa are summarized in Table II, where it will be seen that the inhibition was presented by the extracts of both

TABLE II

Effect of the Extracts Mummy-dried Materials in Distilled Water*

1:	100	200	400	800	1600
Gastric.	##	##	#	+	–
Normal gastric mucosa	##	#	–	–	–

* Explanation for signs, in Table I.

materials, but the gastric carcinoma extract was about 4 times as inhibitory as the normal gastric mucosa extract.

4. *Effect of the extract of a dried tissue in* 2.5 *per cent saline*

Similar tests with the extracts in 2.5 per cent saline instead of distilled water yielded the results quite similar to those with the aqueous extracts, as

shown in Table III.

TABLE III

Effect of the Extracts Mummy-dried Materials in 2.5% Saline

1:	100	200	400	800	1600
Gastric carc.	##	#	+	±	−
Normal gastric mucosa	#	+	−	−	−

5. *Differences in the intensity of heart inhibition according to the form of carcinoma*

The intensity of heart inhibition of gastric carcinoma extracts was found to differ according to the form of the carcinoma, for example, the highest dilution at which the inhibition was still perceptible varied from 1 :800 to 1 :20000 as shown in Table IV,carcinoma solidum gelatinosum and carcinoma adenomatosum gelatinosum presumably being the most effective two.

TABLE IV

Differences in the Intensity of Heart Inhibition according to the Form of Carcinoma

Case	Highest dilution*	Form of carcinoma
1	800	Carc. adenomatosum simplex
2	1600	Carc. solidum diffusum
3	1600	Carc. solidum diffusum
4	5000	Carc. solidum alveolare
5	6000	Carc. solidum gelatinosum
6	20000	Carc. adenomatosum gelatinosum

* Highest dilution at which an inhibition of degree (see Table I) is perceptible.

Summarizing the results of the experiments described in this section it will be concluded that all kinds of extracts of gastric carcinoma, fresh and dried, presented nearly the same order of heart-inhibiting effect, which was about 4 times or more as strong as that of normal gastric mucosa.

Heart-Inhibiting Effect of the Dialyzate of a Crude Extract

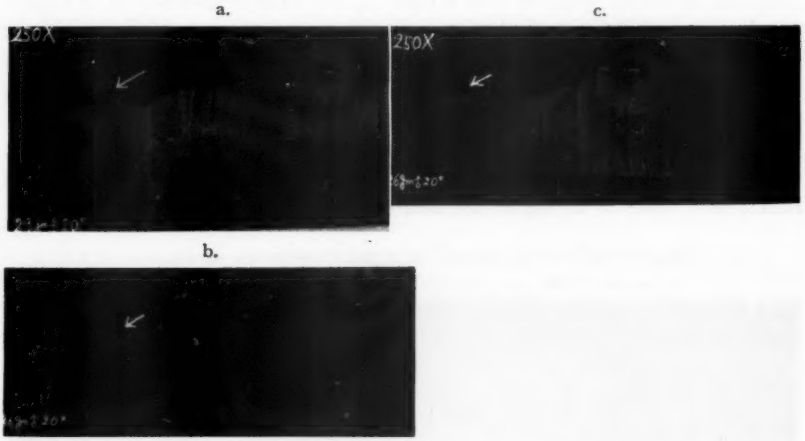
With a view to tracing the heart-inhibiting substance in the crude extracts of gastric carcinoma, the extracts of fresh gastric carcinoma and normal gastric mucosa (10 g. in each case) in Ringer's, 2.5 per cent saline, and distilled water were dialyzed across the cellophane membrane for 48

hours, and the dialyzates and the dialyzed extracts were tested for the inhibiting effect.

1. *Tests of the dialyzed extracts*

In the cases of the extracts in Ringer's and 2.5 per cent saline, each dialyzed extract was centrifuged to remove the preciptitate, and the supernatant was tested for the heart-inhibiting effect at varying dilutions, with the results that neither the gastric carcinoma extract nor the normal gastric mucosa extract showed inhibition, and indeed the effect of the former was stimulatory, not inhibitory, at a dilution of 1 :250 (Fig. 3 a.). When these

Fig. 3. Effect of a gastric carcinoma extract, dialyzed against Ringer's, a) supernatant, 1 :250. b) sediment rinsings, 1 :1000. c) alkali-soluble sediment, 1 :250.



supernatants were condensed and tested, there were often found heart stimulation but never inhibition.

Next, the sediment obtained by the above centrifugation of each dialyzed extract was rinsed repeatedly with distilled water, and the rinsings were made into the isotonic Ringer's and tested for the effect on the heart, with the results that a slight stimulation, not inhibition, was shown (Fig. 3. b.). Furthermore, Ringer's was added to the sediment, and the pH was adjusted to 7.6 with N/10 sodium hydroxide to dissolve the sediment. The solution was then centrifuged, and the supernatant, which was alkali-soluble, was tested. In this case there appeared a slight inhibition (Fig. 3. c.). The extracts of normal gastric mucosa were treated in a similar manner, but showed no inhibition.

Summarizing the results described in this section, it may be concluded that each extract of gastric carcinoma and normal gastric mucosa lost its

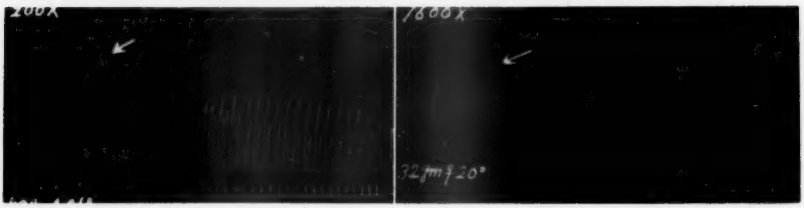
inhibiting action after the dialysis.

2. *Tests of the dialyzates*

In testing the dialyzates, only the aqueous extracts of gastric carcinoma and normal gastric mucosa were used to exclude a possible effect of the salts of the solution themselves against which dialysis was done. Thus, an extract obtained when 90 cc. of distilled water was added to 10 g. of a tissue was dialyzed and the dialyzate was collected. The sac containing the extract was then immersed in new distilled water for further dialysis and after a given period the second dialyzate was collected. By repeating this many times a total of 800 to 1000 cc. of the dialyzate was obtained. This was condensed in vacuum so as to contain solid matter 1 per cent (i.e., 1 :100 dilution), and was tested for the effect on the heart at its doubling dilutions (diluted with Ringer's), with the results shown in Figure 4, where it will be seen that there were presented remarkable inhibition at 1 :200 to 1 :800, slight inhibition at 1 :1600 and 1 :3200, and no inhibition at 1 :6400. As for the dialyzate of normal gastric mucosa there were presented remarkable inhibition at 1 :200 and 1 :400, slight inhibition at 1 :800, and no inhibition at 1 :1600, as shown in Figure 5. Thus it may be seen that the dialyzate of a gastric carcinoma extract presented an inhibition 2 to 4 times as strong as the inhibition of normal gastric mucosa.

Fig. 4. Effect of the dialyzate of a gastric carcinoma extract.
a. 1 :200 b. 1 :6400

Fig. 5. Effect of the dialyzate of a normal gastric mucosa extract.
a. 1 :200 b. 1 :1600



Tracing of the Heart-inhibiting Substance

As the presence of some heart-inhibiting substance in the dialyzate

of an extract was demonstrated, the next step was to examine the nature of this substance by submitting it to various chemical treatments and testing the products for the effect on the heart.

1. *Effect of dryind*

After being tested for the heart inhibition, the dialyzate of a gastric carcinoma extract was condensed to a syrup, and dried in vacuum. Not a perfectly dry but a viscous lipoid substance was obtained. To 20 mg. of this dried material was added 2 cc. of Ringer's to make a 1 :100 dilution, and the solution was tested for the effect on the heart at doubling dilutions (diluted with Ringer's), with the results shown in Table V, where it will be seen that drying exerted no influence on the intensity of heart inhibition.

TABLE V

Effect of Drying on the Intensity of Heat Inhibition
(Material: Gastric Carcinoma, dried; dialyzate)

1:	200	500	1000	2000	4000
Before drying	##	##	+	+	−
After drying	##	##	+	+	−

2. *Effect of heating*

With 10 mg. of the above dried material a 1 :700 dilution was prepared. Some of the solution was used for testing for the inhibition. The remaining solution was heated for 10 minutes at 80°C. or 100°C., and tested at varying dilutions. However, there were found no differences in their effect between the materials before and after the heating as shown in Figure 6.

Fig. 6. Effect of the dialyzate of a gastric carcinoma extract, 1 :700, 80°C., 10 minutes heating.

3. *Effect of treating with a strong acid*

To 10 mg. of the dried material was added 2 cc. of Ringer's to make a 1 :200 dilution, to which was added 3 drops of N/10 hydrochloric acid. After the solution was let to stand for 15 minutes, the pH was adjusted to 7.0 with N/10 sodium hydroxide. Tests with this solution yielded the results shown in Table VI that the treatment with the acid brought about

TABLE VI

Effect of a Strong Acid on the Intensity of Heart Inhibition
(Material: gastric carcinoma, dried; dialyzate)

1:	200	400	800	1600	3200
Strong acid	++	+	±	−	−
Control*	+++	++	+	+	−

* Doubling dilutions of a 1 :100 dilution of the dialyzate of the extract of dried material.

a slight decrease in the intensity of heart inhibition.

4. *Effect of treating with the ion exchange resin*

a) Cations. An amount of 70 mg. of the dried material was dissolved in 2 cc. of distilled water, to which was added 5 g. (wet weight) of cations (Amberlite/I R 120). The mixture was shaken for 20 minutes. To the supernatant was added rinsings that had been obtained by rinsing ion exchange resin 3 times with 5 cc. of distilled water. After the pH had been adjusted to 7.0, the solution was condensed in vacuum to dryness. The dried material thus obtained was tested at varying dilutions, with the results, as shown in Fig. 7, that the inhibition was slight at 1 :25 and 1 :50, and negative at 1 :200.

b) Anions. An amount of 70 mg. of the dried material was treated with 10 g. (wet weight) of anions (Amberlite/IRA 400) in the same manner as before. After the treatment, the material showed a strong inhibiting effect, the effect being perceptible even at 1 :900 (Fig. 8).

Fig. 7. Effect of the dialyzate of gastric carcinoma, passed through cations.

a. 1 :25 b. 1 :200

c) Cations and anions. When the material was treated first with cations, then with anions each for 30 minutes in the same manner as before, it showed no inhibiting action even at 1 :30 (Fig. 9).

From these results it may be concluded that the heart-inhibiting substance was absorbed mainly by cation exchange resin, and when passed through both ion exchange resins the substance was absorbed completely.

Fig. 8 Fig. 9

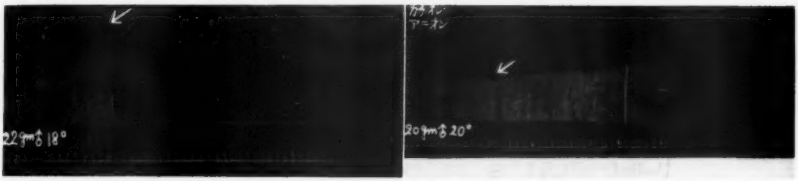


Fig. 8. Effect of the dialyzate of gastric carcinoma, passed through anions. 1 :25.

Fig. 9. Effect of the dialyzate of gastric carcinoma, passed through cations and anions, 1 :30.

5. *Effect of treating with organic solvents*

a) Alcohol. i. 70 per cent alcohol, room temperature. To an amount of 40 mg. of the dried material was added 20 cc. of alcohol, and the mixture was agitated for 30 minutes and centrifuged. The sediment was repeatedly rinsed with alcohol, and the rinsings were united with the supernatant. The solution was condensed in vacuum to dryness, redissolved in Ringer's. The above sediment was dried in vacuum, and redissolved in Ringer's. The two preparations thus obtained were tested for the effect on the heart and were found to show nearly equal degrees of inhibition at the same concentration.

ii. 99 per cent alcohol, room temperature. To an amount of 20 mg. of the dried material was added 20 cc. of 99 per cent alcohol, and the mixture was agitated for 30 minutes, and then centrifuged for 10 minutes at 3000 rpm. The supernatant was dried in vacuum to powder. This powder was tested at varying dilutions, and it was found (Table VII) that the material had a remarkable inhibition on the heart, the effect disappearing first at 1 :1600.

iii. 99 per cent alcohol, boiled. To an amount of 20 mg. of the dried material was added 50 cc. of 99 per cent alcohol, and the mixture was agitated for 10 minutes, boiled for 10 minutes at 100°C., and filtered quickly. The filtrate was condensed in vacuum, and redissolved in 2 cc. of Ringer's to make a 1 :100 dilution. The inhibiting action of this preparation was so strong as to be still fairly manifest at 1 :1600.

b) Ether. The inhibiting substance was found not to dissolve in ether (Table VII).

c) Chloroform. The inhibiting substance was found to dissolve well in dehydrated chloroform (Table VII).

d) Benzole and acetone. The inhibiting substance slightly dissolved in benzole, but not in acetone.

Summarizing all these results given in tihs section, it may be said

TABLE VII

Effect of Treatment with Organic Solvents
(Material: gastric carcinoma, dried; dialyzate)

1:	100	200	400	800	1600	3200
99% Alcohol, room temperature	++	++	+	±	−	−
99% Alcohol, boiled	++	++	+	+	+	−
Ether	−	−	−	−	−	−
Chloroform	++	++	++	+	−	−
Benzene	+	±	−	−	−	−
Acetone	−	−	−	−	−	−
Control	++	++	++	+	+	−

Control: see Table VI.

TABLE VIII

Qualitative Tests of Fractions of the Heart-inhibiting Sustance

		Biuret reaction	Molisch reaction	Bial reaction	Dische reaction
1)	Gastric carc.	++	++	++	+
	Normal gastric mucosa	++	++	++	+
2)	Gastric carc.	+	++	++	+
	Normal gastric mucosa	++	++	++	+
3)	Gastric carc.	+	+	+	−
	Normal gastric mucosa	+	+	+	−
4)	Gastric carc.	−	−	−	−
	Normal gastric mucosa	−	−	−	−
5)	Gastric carc.	−	±	−	−
	Normal gastric mucosa	−	±	−	−

that the inhibiting substance in the dialyzate of an extract is not affected by drying to powder, slightly weakened by a strong acid, absorbed by ion exchange cation, and easily soluble in alcohol and chloroform.

Qualitative Tests of Fractions of the Heart-inhibiting Substance

Biuret, Molisch's, Bial's, and Dische's tests of gastric carcinoma and normal gastric mucosa were performed on their 1) aqueous extracts, 2) dried materials of the dialyzates, 3) extracts in 99 per cent alcohol, 4) extracts by boiling in 99 per cent alcohol, and 5) extracts in chloroform (extracted twice), with the results shown in Table VIII. All the solutions

were tested at a dilution of 1:100. It will be seen that between gastric carcinoma and normal gastric mucosa there were no significant differences in the four qualitative tests of both extracts and dialyzates, and that the alcohol and chloroform solutions of the heart-inhibiting substance yielded only very weak or no positive results in the four tests. From these it may be assumed that the heart-inhibiting substance are negative to these qualitative tests. In addition, tests for hemolysis and phosphorus were made on the extracts by boiling in 99 per cent alcohol, which presented the most remarkable inhibition, but with negative results.

Summary

1. The extract of gastric carcinoma in distilled water, Ringer's solution for frogs or 2.5 per cent saline presented an inhibiting effect on the excised frog heart more than four times as strong as that of the extract of normal human gastric mucosa when studied by the kymographic method.

2. When the extract was dialyzed across the cellophane membrane for 48 hours the heart-inhibiting substance was demonstrated in the dialyzate, not in the dialyzed extract.

3. Various chemical and physical treatments were submitted to this substance to study its nature, and it was found that the inhibiting substance was absorbed by cation exchange resin, and soluble in alcohol and chloroform.

In conclusion, I wish to express my sincere gratitude to Assistant Professor K. Ishizuka of the Pharmacological Institute and to Dr. H. Kawasaki of the Medicochemical Institute of Tohoku University.

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Alkaline Phosphatase in " Congenital Gigantism of Peroxidase Granules "

By

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(Received for publication, January 19, 1955)

The alkaline phosphatase within the normal neutrophils tends, as is well known, to be diffusely distributed in the cytoplasm rather than segregated, for example, in the neutrophilic granules. In other words, the neutrophilic granules do not give the " alkaline " phosphatase reaction (Cf. Jones,[1] Rheingold and Wislocki,[2] and Rabinovitch[3]).

Now, Higashi,[4] one of us, published in this Journal a case of peroxidase abnormity for which the name: " Congenital Gigantism of Peroxidase Granules " was suggested. A hematologist with much experience in peroxidase reaction of leucocytes could never have imagined such an abnormal distribution or rather conglomeration as was presented in the myeloic leucocytes of this strange case. And it was a congenital and familial abnormity, though we shall not go into details here. The alkaline phosphatase picture in this case will be described below.

The alkaline phosphatase activity was examined by Wachstein's[5] method. The marrow specimens used for this examination were about three years old smears; notwithstanding immediately after treatment with ammonium sulfide, some of the slides took a dark color. Under the microscope, a fairly large proportion of cells, almost exclusively neutrophils, was found to contain moderately large amount of the enzyme. The cytoplasm of neutrophils was stained, generally speaking, diffusely black in various intensity. Some of polymorphonuclears and juvenile neutrophils showed very strong alkaline phosphatase activity and the whole cytoplasm was so diffusely and simply blackened that a detailed change could not be seen. Not infrequently, however, especially late myelocytes and polymorphonuclears exhibited, in addition to diffuse cytoplasmic staining, a very coarse brown-colored granulation. The granules were not clearly defined; only careful examination could reveal the presence of somewhat

spherical, large and dark granules in less dark background.

On the other hand, however, in some other neutrophils, especially in intermediate and early myelocytes, the cytoplasm showed a moderate or slight degree of alkaline phosphatase activity, and it was recognizable that they contained some unstained, refractile, large spherical granules, which stood out against the more or less dark brownish background.

Some early myelocytes, however, had multiple, distinctly stained and clearly defined rounded mass in the cytoplasm, while the rest part of the cell was entirely unstained. In some myeloblasts also, weak alkaline phosphatase activity was present in the extravacuolar space of cytoplasm. And some other myeloblasts had a single large vacuole with only partially brownish stained surface. These findings seemed to be particularly interesting in the present case, because these large cytoplasmic stained granules or/and unstained vacuoles seemed to correspond to the sites of gigantic peroxidase granules.

On the basis of above described observations, it seemed likely in this particular case that there were two different ways of the cytological localization of the alkaline phosphatase. (Before further description, the reader will please understand that, in the case of congenital peroxidase abnormity, the oxidase as well as the peroxidase granules occupied the same sites, as was shown and illustrated in Higashi's[4] paper.) In one way of these, alkaline phosphatase was diffusely scattered in the extra- or inter-granular space, not covering the sites of peroxidase (or oxidase) granules. And in the other, the enzyme found itself segregatedly on the sites of peroxidase, apparently so closely associated with peroxidase (or oxidase) granules.

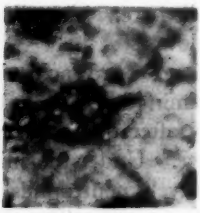

Fig. 1

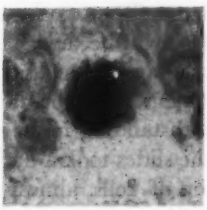

Fig. 2

Fig. 3

Alkaline phosphatase of neutrophils

Fig. 1. The cytoplasm is alkaline phosphatase-positive and contains many alkaline phosphatase-negative coarse granules in it.

Fig. 2. There are a few very coarse granules—strongly alkaline-phosphatase-positive granules in the cytoplasm, and the remaining part is slightly positive in the enzyme activity.

Fig. 3. The cytoplasm is diffusely stained showing the presence of a large amount of alkaline phosphatase. Note the moss-eaten shaped, unstained part, which is probably the site of a chain of gigantic peroxidase granules.

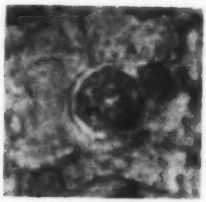

Fig. 4

Alkaline phosphatase of eosinophil

Fig. 4. Alkaline phosphatase is apparently negative in the cytoplasm.

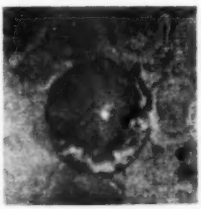

Fig. 5

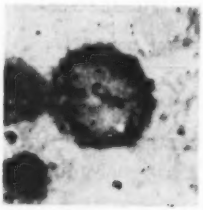

Fig. 6

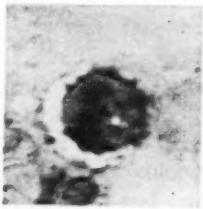

Fig. 7

Alkaline phosphatase of myelocytes

Fig. 5 and 7. Note that the granules are alkaline phosphatase-negative.

Fig. 6. Many alkaline phosphatase-negative granules are contained in the cytoplasm, whereas this is diffusely stained showing the enzyme activity.

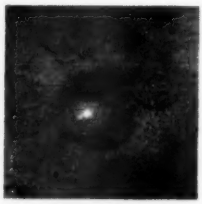

Fig. 8

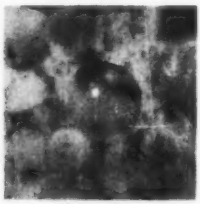

Fig. 9

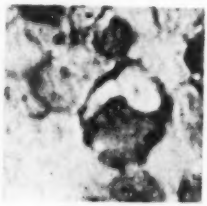

Fig. 10

Alkaline phosphatase of myeloblasts

Fig. 8. The cytoplasm contains a single large vacuole with alkaline phosphatase in only peripheral and superficial parts.

Figs. 9 and 10. Weak alkaline phosphatase activity is present in the extravacuolar space of the cytoplasm.

Summary and Conclusions

"Congenital Gigantism of Peroxidase Granules" is a congenital and familial disease described by Higashi, one of the authors. This monstrous abnormity of peroxidase granules would have been called "Congenital Abnormity of Alkaline Phosphatase", if this enzyme had been the very

first object of our investigation. So abnormal and strange was the distribution of the alkaline phosphatase in leucocytes in this peculiar disease.

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Retinal Induction in Various Regions of Human Retina

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Introduction

The mechanism of contrast has long remained one of the great outstanding difficulties in formulating an acceptable theory of color vision. Motokawa[1] discovered a physiological correlate of contrast and termed it "retinal induction." This phenomenon has hitherto been studied extensively by Motokawa and his associates[1)-5)] on man as well as on animals, in which action potentials of optic nerve or spike potentials from ganglion cells of the retina were used as indicators for determination of electrical thresholds. However, we have not yet any detailed information available of retinal induction in the peripheral retina. Comparison of the fovea with the periphery concerning retinal induction seems to be very important, for it is generally known that contrast occurs more strongly at the mid-periphery than at the fovea. The present investigation aimed at a more extensive survey of this phenomenon all over the retina.

Experimantal

Method

The method is described in detail in previous papers,[6)-7)] so that only the principle of the method for determination of electrical thresholds of the eye will be mentioned here. The method consists in measuring the electrical excitability of the dark-adapted eye with a constant current of 0.1 second, taking the least perceptible phosphene as an index, in order to measure an increase in electrical excitability after a conditioning photic stimulus. Percentage increases of electrical excitability are expressed in terms of which is defined by the formula: $\zeta=100\ (E-E_o)/E_o$, where E and E_o represent electrical excitability determined with and without pre-illumination, respectively.

A circular disc of opaque glass of 1° in visual angle illuminated from behind with spectral light from a spectroscope served as a target. The spectrum used in this experiment was an equal-energy spectrum. The intensity level of the spectrum was given in terms of physiological units. One unit was an intensity 350 times as high as the threshold intensity measured at the dark-adapted fovea with red light (650 mμ) of 2° in visual angle.

The intensity was adjusted to the equal-energy by controlling the width of the collimator slit of the spectroscope to the prescribed values computed from the known spectral energy distribution of the light source.

The intensities of the inducing and the test lights were controlled separately by means of neutral wedges.

Other procedures in experimentation will be described together with the result.

Results

The ζ-time curves for various spectral lights are illustrated in Fig. 1, in which ζ values are plotted as ordinates against time in seconds after the

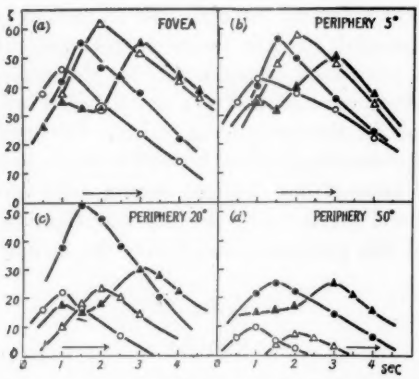

Fig. 1. ζ-time curves obtained at various parts of retina. Ordinates: percentage increases of electrical excitability above resting level. Abscissae: time in sec. after end of pre-illumination with colored light. Curves marked by empty circles, solid circles, empty triangles and solid triangles refer to red (650 mμ), yellow (585 mμ), green (530 mμ) and blue (470 mμ) lights respectively. Duration of pre-illuminating light was 2 seconds.

end of illumination as abscissae. In these experiments, ζ values were measured at varying intervals after 2 seconds exposure of the eye to various spectral lights of intensity 1 physiological unit. The data obtained at the fovea, 5°, 20° and 50° from the fovea are shown in a, b, c and d of Fig. 1

respectively. The curves marked by empty circles, solid circles, empty triangles and solid triangles were obtained with red (650 mμ), yellow (585 mμ), green (530 mμ) and blue (470 mμ) lights respectively. As can be seen in this figure, ζ-time curves for red, yellow, green and blue lights have a maximum at 1, 1.5, 2 and 3 seconds after cessation of the pre-illuminating lights, and the curve for blue light shows tow maxima at 1 and 3 seconds. The first elevation of the curve for blue light is smaller than the second. As was shown by Motokawa's analysis,[6] the crest time of the ζ-time curve which characterizes the pre-illuminating light depends entirely on the wave-length of light used for illumination. When two photic stimuli, colored and white are presented successively, the ζ-time curve determined as above is different from a curve which would represent summation of the effects of both photic stimuli. Examples obtained at the fovea, 5°, 20° and 50° from the fovea are illustrated in a, b, c and d of Fig. 2 respectively. In this experiment, the white test light of 100 lux illuminated the same retinal region that had been illuminated by the inducing colored light of 1 physiological unit. ζ values were measured at different times after exposure to the successive stimuli, colored and white, fixing the interval between both stimuli at 2 seconds. The exposure time of the test and the inducing stimuli were also 2 seconds. In Fig. 2, the curve connecting crosses is the excitability curve for the white test light

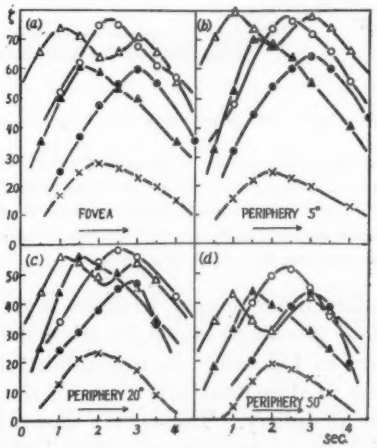

Fig. 2. ζ-time curves obtained at fovea, 5°, 20° and 50° from fovea, with procedure of retinal induction. Brocken curve connecting crosses represents curve for white test light alone. Curves connecting empty circles, solid circles, empty triangles and solid triangles represent curves for red, yellow, green and blue lights each succeeded by the same white light as mentioned above.

alone. As can be seen in the Figure, the curves for the successive stimuli, colored and white, are always higher than that for the white light alone. The curve connecting empty triangles obtained by successive presentation of green and white lights resembles the excitability curve for purplish light, having two maxima at 1 and 3 seconds. Similar measurements were carried out with red, yellow and blue lights combined with the white light. The data obtained are represented by the curves connecting empty circles, solid circles and solid triangles respectively. From these results, it is obvious that the curves for the combined stimuli are complementary in character to the curve which would be obtained for the colored light alone. What has been said about the data obtained from the fovea is equally true of the data obtained from outside the fovea.

In Fig. 3 a, the maximal ζ value of each ζ-time curve shown in Fig. 1 is represented as a function of distance from the fovea. The curves marked

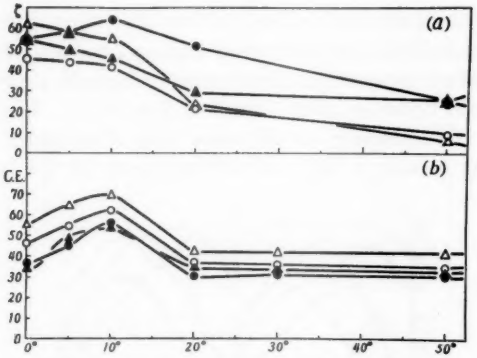

Fig. 3. (a) : Spatial distribution of maximal ζ values of the curves shown in Fig. 1. Abscissae : distances from fovea in visual angle (temporal side of left retina). (b) : Spatial distribution for contrast effects obtained from Fig. 2. Contrast effects mean maximal difference between curve for successive stimuli, colored and white and that for white light alone.

by empty circles, solid circles, empty triangles and solid triangles were obtained by red, yellow, green and blue lights respectively. In accordance with the data obtained by Oikawa,[8] the magnitudes of the yellow and the blue processes become predominant over those of the green and the red processes towards the periphery. The curves shown in Fig. 3 b refer to the spatial distribution of the intensity of retinal induction or the contrast effect, which means the difference between the maximal ζ value of the curve for the successive stimuli, colored and white, and the corresponding value of the curve for the white test light alone. The curves marked by empty circles, solid circles, empty triangles and solid triangles indicate

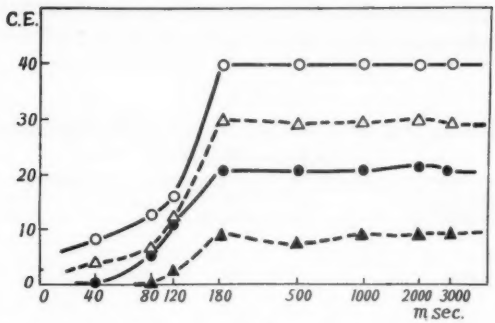

Fig. 4. The dependence of contrast effects on duration of inducing light (585 mμ). Intensity of inducing light was 1 unit for higher two curves and 0.5 for lower two curves. Continuous curves and broken curves were obtained at fovea and 20° in periphery respectively.

regional variations of the contrast effects caused by red, yellow, green, and blue lights, respectively. As can be seen in the figure, the contrast effect is greatest at 10° from the fovea, irrespective of the kinds of inducing lights. It is also to be noted that the contrast effect remains constant in the peripheral retina from 20° to 50° or farther. This relation is common to all sorts of colored lights. Besides, it deserves attention that the contrast effects of red and green lights are greater than those of yellow and blue lights (cf. Fig. 3 a and b).

In the next series of experiments, the influence of various factors such as intensities, duration, etc. upon the contrast effect was investigated. In Fig. 4, the duration of the inducing light (585 mμ) was varied, fixing the duration of the white test light at 2 seconds. The two photic stimuli were separated by an interval of 2 seconds. The intensities of these lights were held constant. The full curves marked by empty and solid circles were obtained at the fovea at different intensity levels of inducing light. The broken curves connecting empty and solid triangles refer to similar data obtained at 20° from the fovea. The higher intensity used was 1 physiological unit and the lower was about 0.5 units. As can be seen in the figure, the contrast effect increased as the duration of the inducing light was lengthened and reached a constant level at the same duration, 180 msec. for the two intensities. In other words, the contrast effect is saturated at 180 msec. beyond which further lengthening of duration has no effect whatsoever.

In the following experiment, the intensity of the inducing light was varied for the fixed test light of 100 lux, the exposure times of the colored and the white lights being fixed at 2 seconds. The interval of both stimuli

was 2 seconds. Dependence of contrast effects upon the intensities of inducing light is shown in Table I.

TABLE I

Relation between the Intensity of Inducing Light and Contrast Effects

Intensity of inducing light Yellow light (585 mμ)	Contrast effect (Fovea)	Contrast effect (Periphery 20°)
0.7^0	39	30
0.7^1	27	18
0.7^2	21	8
0.7^4	4	3
0.7^5	0	0
0.7^7	0	0

Next, the relation between the intensity of the white test light and the contrast effect was studied. The results are shown in Table II. As is

TABLE II

Dependence of Contrast Effects on the Intensity of White Test-Light

	Intensity of test-light in lux	ζ for test-light alone	Intensity of inducing light (0.7°)		Intensity of inducing light (0.7^4)	
			ζ for yellow-white	Contrast effect	ζ for yellow-white	Contrast effect
Fovea	0.1	3	40	37	9	6
	1	5	44	39	10	5
	10	11	50	39	17	6
	50	18	56	38	25	7
	100	23	62	39	28	5
Periphery 20°	0.1	2	32	30	6	4
	1	4	34	30	8	4
	10	7	35	28	10	3
	50	13	43	30	16	3
	100	17	47	30	20	3

shown in this table, the ζ values for the white test light alone increase as its intensity rises. Similarly the ζ values for the successive stimuli, colored and white, increase with increasing intensities of the white test light. In consequence, the contrast effects remain unaltered inspite of the changes of the white test light. This means that the contrast effect is independent

of the test light, but determined by the inducing light. This interpretation will be supported further by the following experiment, shown in Table III,

TABLE III

Dependence of Contrast Effects on the Duration of White Test-Light

Duration of test-light (msec.)	Fovea			Periphery 20°		
	ζ for test-light alone (100 lux)	ζ for yellow-white	Contrast effect	ζ for test-light alone (100 lux)	ζ for yellow-white	Contrast effect
40	15	55	40	12	41	29
80	18	58	40	13	41	28
180	22	62	40	16	45	29
500	22	62	40	17	46	29
1000	23	62	39	17	46	29
2000	23	63	40	17	46	29
3000	23	63	40	17	46	29

in which the duration of the test light alone was varied. The ζ values for the white light alone rose with increasing duration of the test light, and inspite of this increase the contrast effect remained constant. From these experiments it is apparent that the determinant for the contrast effect is the inducing light itself.

Discussion

As has been demonstrated above, the contrast effect is greatest at 10° from the fovea. This fact may be interpreted as a correlate of the well-known psychological fact that color contrast can be perceived more easily in the para-foveal region than in the fovea. Motokawa[3)9)] showed that retinal induction spreads in the surrounding retinal areas with a certain velocity. This fact suggests that the phenomenon of retinal induction would be based on lateral connections in the retina. The lateral connections seem to be lacking at least at the very center of the fovea, but show elaborate development outside the fovea, as a number of histologists such as Cajal, Polyak[10)] and others have pointed out. Although no systematic survey is available on the spatial distribution of lateral connection provided by horizontal cells, amacrine cells, etc., it may be inferred from our experiments that the development of lateral connection would be maximal in the para-foveal and mid-peripheral regions of the retina.

Another aspect worth mentioning of our data is that red and green lights are more effective in producing retinal induction than yellow and

blue lights. Red and green are a pair of complementaries most frequently used in daily life. The pair of yellow and blue is also used, but not so frequently as the pair of red and green. We do not know any scientific reason for this situation, but it would not be far—reaching to suppose that we are utilizing the more effective pair of complementaries, red and green in preference to the other pair, though unaware of its scientific ground.

Summary

By the method of electro-stimulation, retinal induction was measured at various parts of the human retina.

1. The magnitude of retinal induction was found greatest at about 10° from the fovea. This fact might be correlated with the psychological fact that color contrast can most easily be perceived in the para-foveal region of the retina.

2. Red and green lights were found more effective in producing retinal induction than yellow and blue lights.

3. In measurements of retinal induction two photic stimuli are used, i.e., a colored light preceding a white test light. The intensity of retinal induction was shown to be independent of the intensity and the duration of the white test light, but determined by those of the inducing colored light. This law holds good for both foveal and peripheral vision.

The authors are indebted to Prof. K. Motokawa for his invaluable advice and encouragement.

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An Experimental Study to Increase Blood Supply to An Ischemic Myocardium

Part I. Anatomy of Blood Vessels in Canine Heart

By

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It is well known that the blood vessel of the canine heart is different from that of the human beings. Aside from the fairly resembling pattern of the distribution of superficial blood vessel of the mammalian heart[1], the anatomy concerning the distribution of deep vessel has not been studied in details. The author has been particularly interested with the anatomy of the septal artery which has a great relationship with the survival rate with ligation of the coronary arteries of the heart.

EXPERIMENTAL

Method

50 canine hearts were used. After the dog with normal heart was killed with ether inhalation, the heart with the ascending aorta and the brachio-cephalic branch was removed from the body, and thoroughly rinsed with water. A French catheter No. 5 was inserted into the left carotid artery, and the aortic arch and the right carotid artery were both ligated. The coronary arteries were perfused with water for 30 minutes through the inserted catheter with a syringe. Most of the arterial branches were cleared of blood in this manner. Then, another catheter was inserted through the ostium of the coronary sinus and the blood was removed by squeezing of the heart and perfusion. The specimens were immersed in a water bath in a refrigerator 4°C. for 24 hours and the above procedure was repeated. Various measures were used to visualize the vessels in the heart which has almost become white by perfusion. 1) 20 cc. of barium sulphate mixed in water in a ratio of 1:5, or 70% Diodrast was injected into the coronary arteries and the ostium of the coronary sinus. X ray pictures and normal photographs were taken in the anterior-posterior direction with its ordinary position in the body and flattened out position

after incision along the posterior line between the apex and sulcus dividing the ascending aorta and the pulmonary artery. 2) Solution colored with aniline (red) and indigo carmine (blue) was injected into the right and left coronary arteries, respectively, and the distribution of the superficial vessels was delineated. The hearts were also cut in the horizontal plane in several pieces to see the communications between the right and left coronary arteries. 3) After Chinese ink (pigment made of carbon particles) was injected in the arterial and venous system, histologic sections were made to study the fine communications in the deep muscles. 4) In order to study the relationship of the coronary arteries and the veins in situ, various kinds of models were made. At first, celloidin or paraffin was injected into the vessels and found to be inadequate to pursue the fine branches due to their fragility, and it was replaced with the following technique. When methacrylic resin was used, the elasticity, firmness and fluidity were found to be excellent[1]. Briefly explaining the method, ethyl ester of methacrylic acid and its methyl ester are measured separately in a ratio of 1:4–5. The specimen is submerged in a water bath which is kept around 4°C.. Ethyl ester and methyl ester are mixed together thoroughly just before the injection and the mixture is pumped into the coronary systems through the catheter with a syringe. In order to facilitate the interpretation of the relationship of the arterial and venous side, red, yellow and blue oil paints were mixed into the above mixture in about 1%.

Proper attention was taken to prevent water mixing with the mixture. The specimen which was treated in such a way was kept in 20% alkali for 24 hours and later washed with running water to clear the digested myocardium for an hour. Digestion of the heart muscle was often difficult to obtain within a short period of time. It usually took about 4 weeks to complete the procedure by repeating the digestion and washing. It was easy to read the direction, course, division and numbers of the vessels than the aforementioned 3 methods.

Results

The general outline of the coronary arterial system

Two main branches were identified, i.e., the right and the left coronary arteries. The former arised from the right anterior cusp of the aortic valve and coursed along the auriculoventricular sulcus between the right artium and right ventricle. Usually 4 or 6 large branches to the auricular appendage was found besides fine capillaries. One-third of the posterior septum was supplied with arteries from the peripheral branch of the right coronary artery.

The left coronary artery arised from the left anterior cusp of the aortic

valve and divided into the anterior descending branch and circumflex branch after running two or three mm. distally.

(a) *Anterior descending branch*: The bifurcation which usually exists on the postero-lateral side of the pulmonary artery is covered with the left auricular appendage. It courses along the interventricular groove. Usually, 3 to five to the left ventricle and much smaller branches to the right ventricle are found. There is, however, three or four fairly large branch to the left ventricle. They mostly descend over the anterior portion of the left ventricle which has a caliber of two thirds of the main branch. The distribution of this branch supplies one-third of the anterior portion of the left ventricle, anterior region of the septum, and one fifth of the right ventricle. The rest of the left ventricle is supplied by the circumflex branch.

(b) *Circumflex branch*: It courses along the left auriculoventricular groove to the midline of posterior ventricle. Branches half size of the main branch are found 3 to four in number which descend on the left ventricle. The terminal portion of the circumflex branch is named as the posterior descending branch and unites with the anterior descending branch which is supplying the apex. Branches to the left auricular appendage arise directly from the circumflex branch in seven to 10 small twigs which are hard to designate as a sizable branch. Circumflex branch also supplies about one-seventh of the posterior portion of the septum.

(c) *Septal branch*: It arises mostly from the origin of the anterior descending branch and, in a few percentage, from the origin of the circumflex branch and the main stem of the left coronary artery. Though the origin of the septal branch from the anterior descending branch is variable, most of the cases are found arising within 2–3 mm. posterior or postero-lateral to the origin of the main branch. It penetrates the septum perpendicular to the surface of the left heart with two to three fairly large branches distributed evenly on the sagittal plane. Mainly the septum is provided with blood from the septal branch with small blood supply on its marginal portion from the right coronary artery and the circumflex and anterior descending branches. (Figs. 1 and 2).

The general pattern of the coronary venous system

Injections of the right coronary venous system were not met with success. Main outlet for the venous return to the right heart was not known except evenly distributed veins accompanying the right coronary arterial system was found on the surface.

The venous return of the left ventricle was mostly from the coronary sinus. Five or seven fairly large size branches were noticed. Those which were consistent were the posterior ventricular branch, the lateral ventricular branches and the anterior great cardiac branch naming the branches closer to its outlet. The venous network was more finer than the arteri-

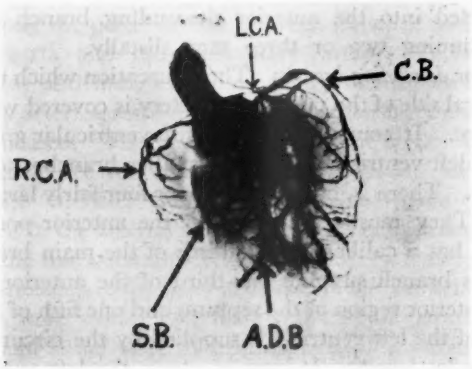

Fig. 1. X ray picture by barium paste showing arterial system of the canine heart. L. C. A.: left coronary artery. C. B.: circumflex branch. A. D. B.: anterior descending branch. S. B.: septal branch. R. C. A.: right cornary artery.

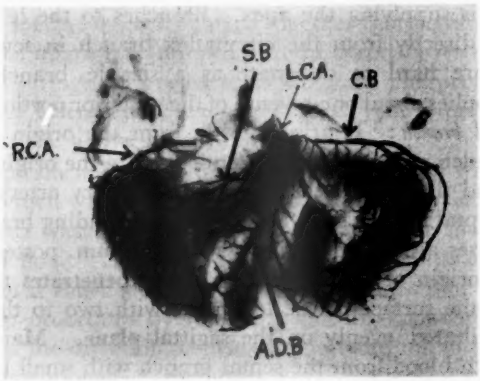

Fig. 2. Canine heart opened in the sagittal plane. L. C. A.: left coronary artery. C. B.: circumflex branch. A. D. B.: anterior descending branch. S. B.: septal branch. R. C. A.: right coronary artery.

al side. The veins usually accompanied the arteries.

Special circulation in the myocardium

Extremely fine anastomoses were found between the arterial and venous system (Fig. 3). Direct communication between the venous system of the myocardium with the ventricular cavity was noticed in the left ventricle. These direct communications were found in the anterior descending branch and the circumflex branch. (Fig. 4).

Fine communications between the branches of the right and left

Fig. 3. Photograph of model by acrylic resin. Abundant fine anastomoses between arteries and venes are seen.

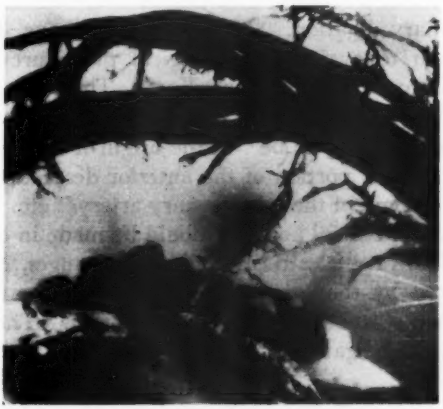

Fig. 4. Photograph showing Thebesian vessels. Top : anterior descending branch and great cardiac vein. Bottom : left ventricular cavity.

coronary arteries and its subdivisions were demonstrated. Chinese ink injected into the coronary artery under pressure of 100 mg. Hg. were not only seen in the capillaries in the deep muscle, but between the space of the muscle fibers (Fig. 5).

Discussion

The general pattern of the coronary system of the dog heart has been presented by others[2)3)], and was confirmed. Present study was to investigate the distribution of the left coronary artery to clarify some of the inconsistent results reported by several investigators on the effect of ex-

Fig. 5. Photomicrograph of myocardium injected with China ink. Left upper corner are capillaries. China ink seen between the muscle fibers.

perimental coronary ligation. Most reporters have tied the anterior descending branch to evaluate the surgical procedures designed to increase collaterals to ischemic myocardium. They, however, only gave obscure statement on the postion of ligation of the coronary arteries. For instance, the ligature is said to be " at the origin of the anterior descending branch ", " the middle portion of the anterior descending branch ", and " 2 mm. from the origin of the left coronary artery " etc. If septal branch is included in the tie, careful analysis should be made in case of interpretation of the survival rate, electrocardiographic changes, etc. The septum is mainly irrigated by the septal branch which arises from the anterior descending branch within a perimeter of 2 mm. Therefore, inclusion of septal branch in the ligature is conceivable. This point should be made more clear.

The size and form of the blood vessels were not given. It is already agreed that the technique of injection greatly changes their conditions in a removed heart. Vein wall are particularly distensible. In spite of pressure of injection was kept around 100 mm. Hg., measurements were quite different from one specimen to another.

Concerning the distribution of the vessels in the myocardium, the description by Grant[4], Batson *et al.*[5] was confirmed. Coronary venous system penetrating the myocardium ended in Thebesian vessels with outlets in the ventricular cavity. Diagramatically, the veins on the surface of the heart were connected to a capillary mesh which lied in the middle of the myocardium and this was bridged with the so called sinoluminal vessels with the ventricular cavity. Direct communication with the arterial system and the ventricle, however, could not be demonstrated.

SUMMARY

The blood vessels in 50 fresh canine hearts which were removed from the body were studied. Various injection methods were used to outline its distribution. Models using methacrylate were found to be the best to obtain the relationship of the vessels. Importance of the anatomy in case of studying the effect of ligation of the vessels were emphasized.

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Fig. 5. Photomicrograph of myocardium injected with China ink. Left upper corner are capillaries. China ink seen between the muscle fibers.

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An Experimental Study to Increase Blood Supply to an Ischemic Myocardium

Part II. Surgical Procedures to Produce Collateral Circulation to Canine Heart*

By

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(Received for publication, February 2, 1955)

It is generally accepted that angina pectoris is caused by myocardial ischemia. Various surgical procedures have been proposed to 1) increase the intercoronary anastomosis of the myocardium[1], 2) dilate the coronary bed by interruption of the coronary constrictors[2], or 3) provide blood from the extracardiac sources[3][4][5]. Despite excellent results claimed by certain reporters, their results have not been confirmed by others. It seems worthwhile to reproduce certain procedures and evaluate the results for its clinical application.

Experimental

Principle of Methods

For production of extracardiac collateral circulation, 1) sterilised talc powder was introduced into the pericardial sac[4]. 2) great omentum[6] or 3) left major pectoralis muscle[3] was sutured to the myocardium, 4) the left internal mammary artery was implanted into the heart muscle[7]. In addition to these procedures, either constriction or ligation of he coronary sinus has been carried out to increase the intercoronary anastomosis. Effect of the ligation of the origin of the anterior descending branch of the coronary artery was performed.

For control, different portions of the left and the right coronary artery were ligated and the survival rate was examined. Studies of ECG, and gross and microscopic examinations of the specimens were made.

Experiment

Method—15 dogs weighing 6 to 12 kg. were used. After they were

* Read in part before the 18th General Meeting of the Japan Circulation Society, Nagoya March 31, 1954.

anesthetised with morphine, the standard leads of the ECG was taken with an ink-writing electrocardiographic machine. With intravenous anesthesia with barbiturates, endotracheal tube was inserted to maintain positive pressure. The tube was connected with a closed circuit anesthesia machine. The operation was carried out aseptically throughout. Thoracotomy was made through the left 5th intercostal space. The right and left coronary arteries were only dissected out without interference with the accompanying veins and a ligature was passed underneath the artery. ECG was taken at frequent intervals during the procedure. Ligation was performed at different site. Those which did not have ventricular fibrillation were permitted to live long to observe the effects.

Results—36 experiments were carried out (Table I). After ligating

TABLE I

Relationship of Ventricular Fibrillation with Ligation of the Coronary Arteries (15 Dogs)

		No. of experiment	Ventricul. fibrill.	
			(+)	(−)
Left coronary artery	Origin	1	3	0
	Origin of circumflex branch	5	5	0
	Origin of ant. desced. branch*	9	6	3
	Periphery to 1st bifurcat. of ant. desced. branch	17	1	16
Right coronary artery	Origin	4	0	4

* Include septal branch.

the left coronary artery immediately after it arised from the ascending aorta (dog. 23), the contraction of the left ventricle became extremly slow with cyanosis and non-contractile heart muscle started to balloon outwards, and the heart was thrown into ventricular fibrillation 1 min. 52 seconds later (Fig. 1. A). The circumflex branch of the left coronary artery was ligated at the bifurcation on five occasions. Ventricular fibrillation was invariably induced within 2 min.–4 min. 30 sec. Ligation at the origin of the anterior descending branch of the left coronary artery was performed on 9 occasions with 6 occurrence of ventricular fibrillation within 3 min. to 26 min. The other 3, however, was followed with ventricular fibrillation within two minutes after the release of its tie. In contrary to the high in-

cidence of ventricular fibrillation from high ligation, ligature at the first division of the anterior descending branch of the left coronary artery for 17 times did not induce ventricular fibrillation but once in dog No. 3 when stiches were passed three times around the vessel to prevent blood loss which occurred during dissection of the vessel. Three animals died 12 hours, 10 days, and 23 days postoperatively due to pulmonary atelectasis, empyema and cardiac failure, respectively. The rest of the animals were used in another experiment after a certain interval. The origin of the right coronary artery was ligated on four occasions without immediate death observed over 20 minutes in each case (Fig. 1. B). The long term observation, however, was not made.

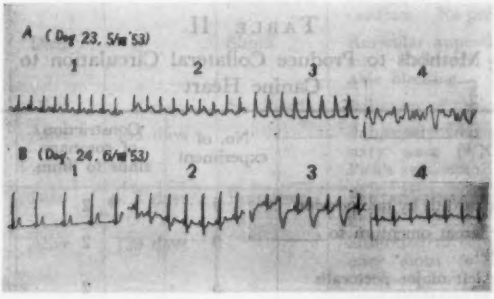

Fig. 1. (A) Ligation of origin of the anterior descending branch of the left coronary artery (Dog. 23, Lead II). 1: preoperative tracing. 2: 16 seconds after ligation. 3: 1 minute 16 seconds after ligation, widening of QRS complex. 4: 2 minutes 20 seconds later ventricular fibrillation.

(B) Ligation of origin of the right coronary artery (Dog. 24. Lead II). 1: preoperative tracing. 2: 15 seconds after ligation. 3: 1 minute after ligation; ventricular extrasystole. 4: 7 minutes after ligation; returned to preoperative record.

Discussion—Ligation of the left coronary artery has produced different results depending on its site. Ligature around the bifurcation of the left coronary artery is, however, not always followed with ventricular fibrillation.[8] Though the single blockage of the septal branch is accompanied with high mortality, survival of the dogs is seen for a quite long period[9]. No study particularly has confirmed whether both septal branch and anterior descending branch of the left coronary at its bifurcation has been ligated or not. The present series of dogs were tested of their patency after the operation. Not a single case escaped from occurrence of ventricular fibrillation when both of them were completely ligated. In discussing the survival rate after ligation of the anterior descending branch of the left coronary artery in the canine heart which has previous operations to in-

crease collateral circulation, the point of ligature should be clearly defined in each animal and confirmed that each dog has the same kind of obstruction of the vessels to evaluate the results in an experiment or to compare the data with others.

Summary—15 dogs have been subjected to ligation of the right and left coronary arteries. Ligation of the main stem of the left coronary artery, origin of the anterior descending branch including the septal branch, and the circumflex branch of the left coronary artery, induced ventricular fibrillation, while ligation at the first division of the anterior descending branch did not cause serious cardiac disability on the heart on 15 occasions.

2) Methods to Increase Blood Supply to the Heart

TABLE II

Methods to Produce Collateral Circulation to Canine Heart

	No. of experiment	Constriction of coronary sinus to 3mm.	Ligation of ant. desced. branch
1. Artificial pericarditis by talc powder	9	2	2
2. Suturing the great omentum to myocardium	9	2	0
3. Suturing the left major pectoralis muscle	5	2	1
4. Implantation of the left internal mammary artery	10	0	1
5. Arterialization of coronary sinus	5	0	0
6. Constriction of coronary sinus	1		1
7. Ligation of coronary sinus	5		2
	44	6	7

(a) *Artificial pericarditis by sterilized talc powder* (Tables III & IV)

Method—After the dogs were anesthetised and positive pressure machine was hooked up as same as the method in the control group, a thoracotomy was made over the left 4th rib, and the heart was exposed by excision of the 4th cartilage. A vertical incision in the pericardium parallel to the left phrenic nerve was made. A small burr was scrubbed over the left ventricle and visceral pericardium followed with spraying 1 gm. of sterilised talc powder into the pericardial sac evenly over the anterior and posterior surface of the left heart muscle. The pericardium was loosely sutured to admit leakage of fluid accumulation and the wounds were sutured in two layers.

After a certain interval, some of the animals were subjected to partial

TABLE III

Results of Canine Heart with Extracardiac Collaterals

No.	Date of operation	Result	Survival	Cause of death	Notes
(I) Talc powder injected in pericaridal sac					
25	25/III	Died	16 hours	Pulmonary atelectasis	Left lung collapsed. Pericardial adhesion slight. Blood vessels of myocardium and pericardium injected.
20	26/III	Died	165 days	Lobar pneumonia	Lung adhesed to pericardium. Dilatation of blood vessels of pericardium. No pericardial effusion.
36	6/V	Died	0	Shock	Auricular appendage accidentally cut. Followed with uncontrollable bleeding.
38	23/V	Died	3 days	Sacrificed	Injection of pericardial vessels.
39	25/V	Alive	133 days		Subjected to constriction of coronary sinus (9/X). No sign of Pick's syndrome.
40	27/V	Died	3 days	Empyema	Pericardial sac obliterated. Bleeding occurred when separated.
41	29/V	Alive	129 days		Subjected to constriction of coronary sinus (6/X). No sign of cardiac failure. Ascites (−). Venous dilatation of skin not observed.
42	30/V	Died	29 days	Sacrificed	Incision into pericardium caused considerable oozing. Part of lung attached to pericardium.
43	1/V	Died	28 days	Sacrificed	Firm pericarditis. Cardiac movement not interfered.
(II) Great omentum attacyd to myocardium					
29	6/IV	Died	8 days	Sacrificed	Eat & run around as healthy dogs from the 2nd postoperative day. Part of the graft twisted & detached from the myocardium
30	17/IV	Died	5 days	Sacrificed	Blood vessels in the graft not abundant, but dillatation of vessels observed.
31	20/IV	Died	11 days	Empyema	Empyema of left pleural cavity. Complete dehiscence of the graft.
32	22/IV	Died	20 min.	Shock	While suturing the graft to myocardium, bleeding occurred. Though controlled, died of shock.
33	21/IV	Died	5 days	Empyema	Inflammatory reaction in the graft and myocardium.

TABLE III—Continued

No.	Date of operation	Result	Survival	Cause of death	Notes
(II) Great omentum attached to myocarcium					
34	7/V	Alive	150 days		Died 2 hours after constriction of of coronary sinus (7/X). Intestine herniated through the diagram.
35	9/V	Died	18 hours	Pulmonary atelectasis	Adhesion between graft & myocardium slight.
44	3/VI	Died	16 days	Empyema	Postoperatively, occasional vomiting noticed. Graft constricted the stomach.
45	4/VI	Alive	130 days		Died 24 hours after constricting the coronary sinus (13/X). Pyloric stenosis caused by graft. Communications between graft & myocardium not abundant.
(III) Major pectoralis muscle to myocardium					
27	28/III	Alive	195 days		Coronary sinus narrowed (12/X). Lived as healthy dogs.
47	12/VI	Died	75 days	Unknown	Adhesion between heart & pericardium. Lung & graft firmly adhesed to myocardium.
48	13/VI	Alive	120 days		Coronary sinus narrowed (11/X). Lived as healthy dogs.
49	16/VI	Died	30 days	Sacrificed	Fibrous graft.
50	18/VI	Died	0	Ventricular fibrillation	When graft stitched to the heart, heart was thrown into ventricular fibrilation.
(IV) Implantation of internal mammary artery into the myocardium					
Group I (Graft implanted into healthy myocardium)					
51	23/VI	Died	6 days	Sacrificed	Pleural exudate not much. Excellent anastomosis between graft & myocardium.
90	17/IX	Died	5 days	Empyema	Graft thrombosed.
907	24/IX	Died	0	Shock	Accidental injury to left auricular appendage caused great blood loss.
916	12/X	Died	7 days	Sacrificed	Graft thrombosed.
917	17/X	Alive	30 days		Graft thrombosed & atrophied. Adhesion of lung & pericardium to myocardium noticed.
920	28/X	Died	16 days	Distemper	Graft patent. Anastomosed with superficial veins of myocardium.

Group II (Graft implanted into ischemic myocardium)

928	28/V	Died	2 months	Empyema	Graft implanted 3 mm. into area of ant. desc. branch after ligation. Graft thrombosed & detached from myocardium.
924	31/V	Alive	7 months	Sacrificed	Living in excellent condition.
925	29/V	Died	7 days	Sacrificed	Graft thrombosed.
921	2/VI	Died	8 days	Empyema	Ant. desc. branch ligated on 2/VI. Graft implanted in infarct on 5/VI. Died on 14/VI. due to empyema. Graft thrombosed.
	(V) Arteriali-ation of coronary sinus				
901	15/IX	Died	0	Shock	Dissection of coronary sinus caused uncontrollable bleeding.
902	16/IX	Died	0	Shock	Ditto
905	21/IX	Died	0	Shock	Ditto
907	24/IX	Died	0	Shock	Ditto
908	30/IX	Operation suspended	Living		Ditto. Due to profuse bleeding, coronary sinus was ligated.
	(VI) Ligation of coronary sinus				
910	24/IX	Died	0	Shock	During dissection of the coronary sinus, massive bleeding occurred. Died 20 minutes after ligation.
908	30/IX	Alive	13 months		No sign of cardiac failure. Refer to Table (4).
19	17/II	Died	0	Shock	Massive bleeding occurred during dissection of the vessel. Died within 24 hours.
909	3/X	Alive	41 days		No sign of cardiac failure. Refer to Table (4).
911	12/VII	Alive	204 days		Living as other healthy dogs. No sign of cardiac failure.
	(VII) Constriction of coronary sinus				
918	19/X	Alive	19 days		Living without any cardiac symptoms.

ligation of coronary sinus in order to increase the deep intercoronary anastomosis; The dogs were tied down on its left side and an incision was made over the left 7th rib. After the pericardium was incised longitudinally, the right auricular appendage was grasped with a Satinsky clamp. The ostium of coronary sinus to the right atrium was usually found 2 mm. or 3 mm. peripheral to the posterior ventricular branch of the circumflex artery. A ligature was passed at this point around a glass cannula with a diameter of 3 mm. lied directly over the coronary sinus and tied securely. Then, the cannula was removed leaving a 3 mm. constriction of the coronary

TABLE IV

Ligation of Origin of Anterior Descending Branch of the Left Coronary Artery

No.	Date of operation	Length of constriction of coronary sinus	Cause of death	Survival	Notes
39	9/X	39 days	Cardiac arrest	23 min.	Pericarditis due to talc powder for 172 days. Dissection caused considerable bleeding.
41	6/X	44 days	Ventric. fibrillation	8 min.	Pericarditis due to talc powder for 173 days. Part of lung firmly attached to pericardium. Dissection caused profuse bleeding.
27	12/X	24 days	Alive	13 month	Left major pectoralis muscle sutured to myocardium for 219 days. Tolerated the procedure well. Living as normal dog.
917			Ventric. fibrillation	2 hours	Internal mammary artery implanted for 30 days & found to be thrombosed. In spite of adhesion of surrounding tissue to myocardium, heart fibrillated.
918	12/XI	19 days	Shock	1 hour	After ligation, temporary cardiac standstill occurred.
908	30/IX	Ligation of the coronary sinus for 13 months	Cardiac failure	6 hours	Dilation of the great cardiac vein. Many veins found over the pericardium. Walked around 3 hours after operation. Died suddenly.
909	3/X	Ligation of the coronary sinus for 41 days	Cardiac failure	40 min.	Many dilation of veins over the pericardium & epicardium. Considerable oozing ensued by incision. Immediately after ligation, heart contraction weakened.

sinus. Due to the interference with the coronary return, the heart engorged and the pericardium had to be left opened. The wound was closed in the usual manner. With both preparations of the dog living for a certain period of time, the origin of the anterior descending branch of the left coronary artery was ligated to test the adequacy of the collateral circulation.

Results—9 dogs were subjected to the experiment. These dogs lived from 16 hours to 5 months. 4 dogs died, of which one died accidentaly from uncontrollable bleeding by cutting the right auricular appendage during incision of the pericardium, and the other three from pulmonary atelectasis, empyema and pneumonia, 16 hours, 3 days,and 5 months later, respectively. Immediately after the operation, the dogs were listless for a couple of day, but regained appetite and started to run around as same as other normal dogs. Neither symptoms of cardiac failure, nor edema formation, or

pericardial effusion were noticed during this period. 2 dogs (Dog 39, & 41) had partial ligation of coronary sinus after 4 months followed with ligation of the origin of anterior descending- and circumflex branch, 39 and 44 days later, respectively. They died due to occurrence of ventricular fibrillation after 23 minutes and 8 minutes, respectively. Gross findings: 16 hours later, the vessels of the pericardium and the epicardium of the left ventricle was injected and dilated with slight adhesion between the visceral and parietal pericardium. Pericardial effusion was slight. About a month later, the process of pericarditis became fairly subsided. The pericardium became fused together with many small capillaries running over its surface. The lung was partly adhesed to the pericardium which caused bleeding when it was incised. Pericardial effusion was, however, in a slight amount; immediately after constriction of the coronary sinus, the veins of the left ventricle became engorged with venous blood and cyanosis ensued. Many non-visible venules prior to operation also became visible. One month after both procedures, dissection into the anterior descending branch caused considerable bleeding. Including the great cardiac vein, many small tortuous branches of veins over the left ventricle were in dilatation. Pericardial sac was completely obliterated with the granuloma (Fig. 2).

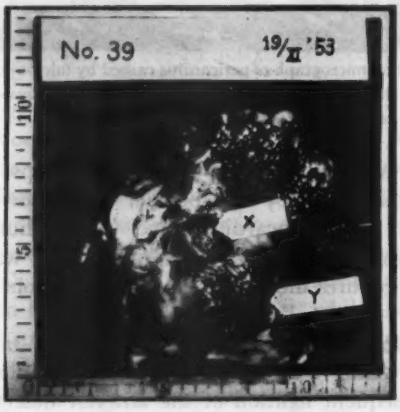

Fig. 2. Talcum powder sprayed in the pericardial sac for 178 days. Well developed granuloma seen. Ostium of coronary sinus constricted to 3 mm. for 41 days. Ligation of origin of anterior descending branch caused cardiac arrest. X: left appendage. Y: talc powder granuloma.

Microscopic findings—A representative specimen of Dog 41 is described. Fine particles of talc powder was found scattered over the myocardium. Fibrocytes and round cell infiltration with few macrocytes surrounded the

foreign bodies. Several sizes of new capillaries were observed (Fig. 3).

ECG—Only lead II was recorded throughout the entire procedure. The most characteristic finding was that ventricular extrasystole was universally found in case of constriction of the coronary sinus, and occasionally low voltage of QRS complex with bradycardia was observed. It, however, disappeared within 24 hours (Fig. 4).

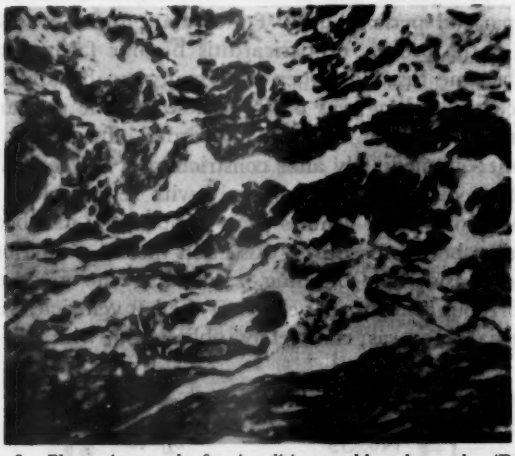

Fig. 3. Photomicrograph of pericarditis caused by talc powder (Dog. 39). Myocardium is shown below. Granuloma caused by talc powder with capillaries is in the center. H. E. stain.

Discussion—As already being reported by Thompson[1], production of artificial pericarditis caused by talc powder was followed with capillary formation between the myocardium and pericardium. Intercoronary anastomosis by coronary sinus constriction, however, was not greatly increased. Such procedures didn't protect the heart from extensive myocardial ischemia.

Summary—9 dogs had pericarditis caused by talc powder. New capillaries were formed. 2 of 9 dogs had coronary sinus constriction after a month with subsequent ligation of the anterior descending branch and septal branch which were fatal.

(b) *Attachment of great omentum to left myocardium* (Tables III and IV)

Method—The great omentum was brought up through the diaphragm and sutured over the anterior and posterior ventricle with 4 or 5 stitches without roughening the epicardium through a thoroactmy in the left 6th intercostal space. The incision to the diaphragm was 3 cm. long in the direction of the muscle fibers. In order to prevent intestinal hernia-

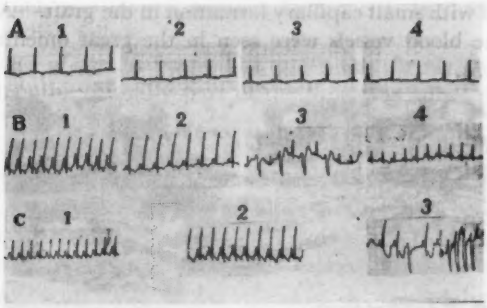

Fig. 4. Electrocardiographic tracing of Dog. 41 with pericarditis caused by talc powder.

(A) Talc powder in the pericardial sac 29/V′ 53. 1 : before the operation. 2 : chest opened. 3 : after roughening the myocardium & talc powder in the pericardial cavity. 4 : chest wound closed

(B) Ostium of coronary sinus narrowed to 3 mm. (6/X′ 53). 1 : before operation. 2 : chest closed. 3 : after coronary sinus constricted. 4 : chest closed.

(C) Ligation of the origin of the anterior descending branch of the left coronary artery (20/XI′ 53). 1 : before the operation. 2 : chest opened. 3 : 1 minute after ligation. Died 8 minutes later by ventricular fibrillation.

tion, few stitches were made between the omentum and diaphragm. Pericardium was loosely closed. Constriction of the cornoary sinus was carried out in the usual manner.

Results—9 dogs were used. The operation was accompanied with various complications. 3 died from pneumothorax 18 hours later, and another died from sudden ventricular fibrillation during closure of the chest wound. 4 lived from 5 days to 5 months. Dog 45 and 34 had constriction of the coronary sinus at its entrance to the right atrium after 4 months and 5 months, respectively; Dog 45 died from shock. It was in a condition of malnutrition due to pyloric stenosis which was caused by the great omentum brought up into the chest, while, Dog 34 was found to have intestine and spleen herniated through the incision in the diaphragm.

ECG—No particular findings were seen from the 1st operation, while constriction to the sinus caused transient ventricular extrasystole and deep negative T.

Gross findings—The graft was fairly well attached to the myocardium after a week. No exudate was found in either the pericardial sac or in the pleural cavity. Complete adhesion was formed between the graft and myocardium after 4 months. Vascularization of the graft was not prominent. Atrophy of the graft was not observed.

Histologic findings—Within a week, the blood vessels in the myocardium

became dilated with small capillary formation in the graft. After 5 months, fairly large size blood vessels were seen in the great omentum and myocardium (Fig. 5).

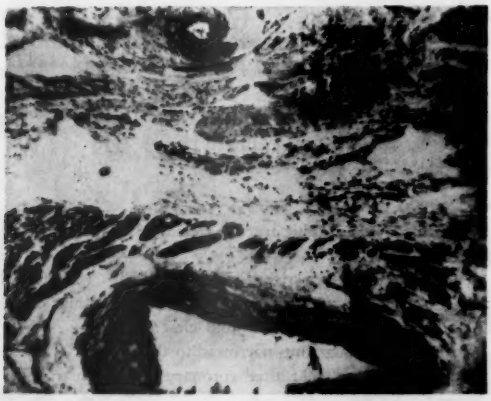

Fig. 5. Photomicrograph of great omentum adhesed to myocardium. Dilated blood vessels in myocardium. Small vessels are shown in the right upper corner. H. E. stain (Dog 45).

Discussion—Complications seemed to be comparatively high from this procedure. Though it is without doubt that new capillaries are formed between the graft and myocardium[1)], additional operation to the coronary sinus didn't increase the vascularity.

Summary—9 dogs were operated, of which 2 had constriction of the coronary sinus. Increase of vascularity was not prominent.

(c) *Attachment of the left major pectoralis muscle to the left myocardium* (Tables III & IV)

Method—A pedicle graft of the left major pectoralis muscle was sutured to the left ventricle. The graft was brought through the excised 4th and 5th left cartilage and stitched to the anterior and lateral side of the left ventricle. The chest wound was carefully covered to prevent pneumothorax. Coronary sinus was narrowed to 3 mm. in 2 dogs, and 1 had ligation of the left coronary artery.

Results—5 dogs were used, of which one died during suturing the graft to the myocardium from sudden occurrence of ventricular fibrillation. 4 dogs lived from 1 month to 22 months. All dogs were in excellent health soon after the operation. Both Dog 27 and 48 were subjected to constriction of coronary sinus after 6 months and a half, and 4 months, respectively. Both dogs tolerated the operation very well and lived as same as healthy dogs. After the second operation in Dog 27, anterior

descending branch was ligated without fatality. It is living over a year without any cardiac failure.

Gross findings of the specimen—The graft was firmly attached to the heart after a month. Usually part of the lung was adhesed either to the graft or to the myocardium. Neither displacement of the heart from its position nor weakening of the heart muscle was noticed. Bleeding from the graft when incised was profuse, but easy to control.

Microscopic findings—Muscle fibers of the graft usually became atrophied after a month with small numbers of capillaries. Infiltration of round cell and fibrocytes was not remarkable. Operation to the coronary sinus had little influence upon the blood vessels in the myocardium (Fig. 6).

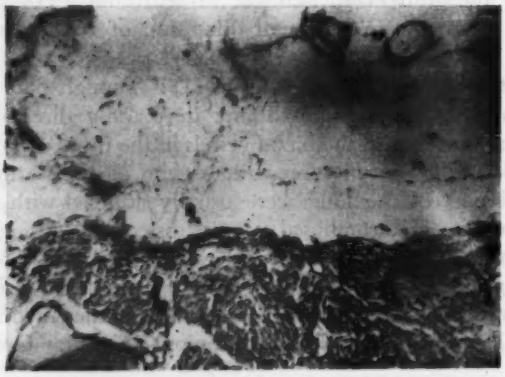

Fig. 6. Photomicrograph with left major pectoralis muscle sutured to myocardium. Capillary with thickened wall seen. (Dog 48) H. E. stain.

Discussion—Beck[1] was impressed by the extracardiac collaterals for the ischemic heart through the sutured graft. According to the histologic sections, the collateral formation didn't seem to be great. In spite of the tedious technique, the operation was surprisingly well tolerated by the dogs.

Summary—2 of 5 dogs with left major pectoralis muscle attached to the myocardium was subjected to constriction of the coronary sinus. One dog tolerated ligation of the anterior descending branch and is living over 22 months.

(d) *Implantation of the left internal mammary artery into the myocardium* (Tables III & IV)

Method—9 dogs were used for the experiment. They were divided into two groups; Group (1): After implanting patent vessel into the myocardium for a certain period, the anterior descending branch was

ligated. Group (2): After ligating the anterior descending branch, the patent vessel was immediately implanted into the left anterior myocardium.

Results—Group (1): 6 dogs were used. All lived from 5 days to one month but one which died on the operating table from great blood loss during accidental trauma to the left auricle. Dog 917 had ligation of the origin of the anterior descending branch after implantation of the graft for one month. It died two hours later due to shock. The graft was found to be thrombosed. Only 3 dogs were found to have patent graft, of which two had excellent communications with the vessels in the myocardium. Group (2): 4 dogs were used. 2 of the three dogs died from empyema after 8 days and 2 months, respectively. One is living 6 months after implantation of the graft in an ischemic myocardium. Postoperatively, no dog died of bleeding. Aneurysmal dilatation of the heart was not observed.

Gross findings of the specimen—Group (1): Though patent vessels were implanted into the myocardium 3 mm. deep, only three were found to have communications with the blood vessels in the myocardium by injection of Indian ink and X ray pictures with barium paste into the graft. Pericardium and part of the lung were usually adhesed with inflammatory reaction. In spite of thrombosed vessels, dissection of the graft en mass from the myocardium caused considerable bleeding. Communications between the graft & myocardium were, however, not in consistency. Some only anastomosed with the superficial vessels in the myocardium. The specimen in Dog 51 had excellent anastomosis (Fig. 7). Group (2): Two

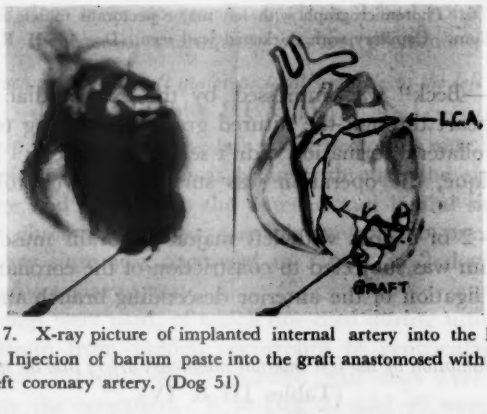

Fig. 7. X-ray picture of implanted internal artery into the left myocardium. Injection of barium paste into the graft anastomosed with branches of the left coronary artery. (Dog 51)

dogs which died showed thrombosis of the graft without communications at all. Dog 925 which had a graft implanted into an infarction produced 3 days previously, died a week later. The graft was also completely

thrombosed. The anastomosis was only found around the place where the graft was implanted.

Microscopic findings—Anatomosis was not established in all experiments. Those with thrombosed graft only showed slight anastomosis around the implanted area. However, excellent communications was established within a week when the graft was patent. In Dog No. 51, patent graft was seen deeply penetrating into the myocardium accompanied with several dilated vessels. The intima of the implanted vessel showed thickening with infiltration of round cells. (Fig. 8). Those with thrombosed vessels had small capillaries around the implanted portion.

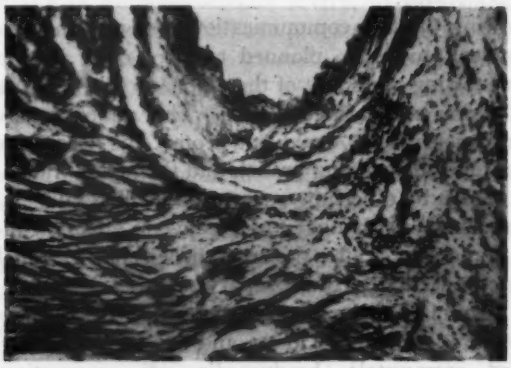

Fig. 8. Photomicrograph of patent internal mammary artery just to enter the myocardium. H. E. stain (Dog 51).

Graft into the myocardial infarct haven't revealed abundant formation of capillaries.

Discussion—High incidence of thrombosis of the graft was recognised. Even with the patent graft, the anastomosis was not always connected with the deep vessels in the myocardium. Complications such as aneurysmal dilatation of the myocardium, bleeding into the muscle, etc., were not found in any case[7].

Summary—6 dogs had implantation of the left internal mammary artery into the left myocardium. Only one of them had anastomosis between the graft and the deep vessels in the myocardium. 3 dogs had graft implanted into the myocardial infarct without evident anastomosis in the infarct.

Comment

General Comment

It is well known that non-functioning anastomosis in dog's heart can

be used as an excellent passage to permit blood flow into an infarcted area under certain circumstances[10]. In our own studies using 2 rabbits, we have ligated the anterior descending branch and injected 2% sodium fluorescein[14] into their ear veins. Though the infarcted area did not become yellow-green while the rest of the heart turned out yellowy, intraarterial injection of blood changed the infarcted area to yellow-green from the periphery demonstrating that anastomosis existed between the arterial trees. It was also proved in 3 dog's heart in the following way: After the anterior descending branch was ligated, blood flowed out from a plastic tube inserted into the distal portion of the ligated vessel when intraarterial injection was performed.

After knowing the fine communications between the artery and vein by the anatomical study, we planned to force open the nonfunctioning channels by narrowing the outlet of the coronary vein[1], and bring in more arterial blood from extracardiac source. This added procedure of constricting the coronoary sinus could not protect the heart from ligating the origin of the anterior descending branch and the septal branch. Reviewing the expectant results by others on extracardiac collaterals[3][4][7], constriction of the coronary sinus was thought to give adverse effect on the circulation. Dog 918 died an hour later after the anterior descending branch was ligated with previous constriction of the coronary sinus 19 days ago. 2 dogs also died within 12 hours after the anterior descending branch was ligated with ligation of the coronary sinus 41 days, and a year, previously. The ECG always showed extrasystole, bradycardia, etc., suggesting myocardial ischemia. (Tables III and IV)

All in all, protection of the heart could not be given by the added procedure of constricting the coronary sinus.

Arterialization of the coronary sinus[11] was unsuccessful. (Table III) It was carried out in 5 dogs. Great amount of bleeding occurred during dissection of coronary sinus. Especially, the veins on the posterior wall caused considerable bleeding and 4 dogs were lost. Stenstrom[12] reported 40 death by merely performing anastomosis between an artery and coronary sinus. It is doubtful whether such difficult procedure is worthwhile to carry out in invalid patients with myocardial ischemia.

Surgical procedures designed to produce extracardiac collaterls as being claimed by others have shown some divergent results. Due to the few numbers of experiment, no statistical evaluation can be deduced. However, according to the simplicity of the procedure and histological studies, pericarditis caused by spraying talc powder in the pericardium seems to be a promising procedure[4].

In addition, if the implanted graft into the myocardium could be kept patent, the collaterals seems to be established fairly well[7].

In all procedures, the lung was always attached to the myocardium. It must be a great help to supply blood to the myocardium[13].

Summary

According to the anatomical study of the canine heart, abundant collaterals were found existing in the heart. In order to open them up and increase the extracardiac blood flow, coronary sinus was constricted to 3 mm. after 1) pericarditis was caused by talc powder, 2) great omentum, and 3) left major pectoralis muscle was sutured to the myocardium. The dogs did not survive the ligation of the origin of the anterior descending branch of the left coronary artery. Either single constriction or ligation of the coronary sinus did not protect the heart from ligating the anterior descending branch. Such procedures to prevent the outflow of of the coronary venous blood do not seem to contribute to increase the intercoronary anastomosis.

Implantation of the left internal mammary artery into the myocardium will make good anastomosis with the heart muscle as far as it is patent. However, the incidence of thrombosis is considerably high.

As dissection of coronary sinus is met with imminent bleeding, anastomosis is quite difficult to establish complete arterialisation of coronary sinus. The procedure cannot be recommended as a safe operation.

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The Effect of Hypotensive Agents on Renal Hemodynamics in Hypertensive Patients

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While most of clinicians attempt to reduce the elevated blood pressure in the treatment of hypertension, it remains still as an important problem to decide whether or not thus artificially lowering blood pressure is advantageous for the hypertensive patients.

Frey,[1] Smith[2] and others maintain that there exists vicious circle between hypertension and arteriolar changes or renal hemodynamic disorders, so that its interruption is recommendable as an excellent treatment of hypertension; according to them the artificial reduction of the blood pressure is favourable to the hypertensive patients. Sassa[3] suggests that in general the continued high blood pressure is harmful and its lowering with some treatments may remove the undesirable effects on the cardiovascular system, consequently the renal vascular damage being prevented to some extent. In this respect he agrees with Volhard's opinion.

Earlier Ito[4] observed in experimental hypertensive rabbits and patients of hypertension apparent decreases of glomerular filtration rate, tubular reabsorption and creatinine excretion when the blood pressure is lowered by giving nitrite salts. From these experimental data, Kato[5] inferred the elevated blood pressure may be a compensatory phenomenon to maintain the adquate blood supply to glomerulus and the sufficient urine volume, so that it is sometimes harmful to reduce the hypertension recklessly. However, he argues from another point of view, the adequate artificial lowering of the blood pressure may be favourable in hypertension, for it often leads to decrease of the renal vascular spasm and improvement of the renal blood flow. Indeed, in hypertension, there is spasm of afferent glomerular arterioles, causing a poor blood supply to glomerulus, consequently the continued hypertension leads to renal damage.

Recent advance in hypotensive agents brought a series of effective drugs, such as hexamethonium salts, veratrum alkaloids, 1-hydrazino-

phthalazine, etc. widely in practical use in hypertensive diseases, but their effects on cardiovascular and renal function are still under discussion, which is chiefly concentrated to the question of their therapeutic usefulness in the treatment of hypertension. Moyer[6], Oshima[7] and others inferred they should be used after measuring the change in the blood flow to various organs. Fishberg[8] and others[12][28] suggested that in the hypertension with severe arteriosclerosis in the important organs, such as heart, brain, kidney, etc., abrupt fall in blood pressure may lead to inadequate blood supply to these arteriosclerotic organs, consequently the artificial reduction of the blood pressure is disadvantageous, while in the absence of such arteriosclerotic complication, the patient will be favoured by lowering the blood pressure. In general, it seems that the effect of hypotensive agents is different in each case, according to the form as well as the severity of the hypertension, so their indication should be determined observing the reactions on each patient.

The purpose of the present investigation is to study the acute response of renal hemodynamics in hypertensive patients when the blood pressure is artificially lowered, using some hypotensive agents, because in hypertension the changes of renal hemodynamics play the most important role at every stage of the disease. An attempt is made to find the difference, if any, of the hemodynamic response between those agents, further between each stage of essential hypertension and renal hypertension, and to discuss on the above problem.

Experimental

Methods

Hexamethonium bromide, veratrum viride and 1-hydrazinophthalazine were injected intramuscularly or intravenously in 23 cases, including essential hypertension, malign hypertension and renal hypertension. The hemodynamic changes in kidney following the lowered blood pressure induced by these drugs were observed by measuring glomerular filtration rate (GFR) (mannitol-clearance), renal plasma flow (RPF) (*p*-aminohippuric acid-clearance), filtration fraction (FF) and renal vascular resistance (RVR), previously one or two times during 45 minutes before injection, and two or four times between 15 and 60 minutes after the injection of the hypotensive agents. The clearances were estimated by constant infusion technique.[9],[10] Estimations of *p*-aminohippuric acid (PAH), and mannitol were performed by the method of Page and Corcoran[11] with spectrophotometer after deproteinisation by Fujita-Iwatake's method.[11] GFR, RPF, renal blood flow (RBF), FF and RVR were calculated as follows:

GFR (cc/min.)=infused mannitol per min/concentration of mannitol in plasma,
RPF (cc/min.)=infused PAH per min/concentration of PAH in plasma,
RBF (cc/min.)=RPF/100—hematocrit reading,
FF=GFR/RPF,
RVR=MAP/RBF.

Blood pressure was determined at frequent intervals with Riva-Rocci's sphygmomanometer, and mean arterial pressure (MAP) was calculated as the third pulse pressure plus diastolic pressure. These values of clearance were corrected for standard body surface area 1.48 in sq. m. Throughout the experiment, the patients remained in the recumbent position to eliminate postural effects on blood pressure and on kidney function.

Classification of essential hypertension was made according to Schroeder[12]: I stage (mild benign hypertension), II stage (moderate benign hypertension), III stage (severe benign hypertension) and IV stage (malignant hypertension).

Results with Discussion

Renal hemodynamics in normotensive persons

Renal hemodynamic values of normotensive adults including 3 male and 2 female were estimated (Table I). Those values indicate an average with its reliable limit ($\alpha=0.05$).

Renal hemodynamics in hypertensive patients

In 25 hypertensive subjects of several forms the renal hemodynamic values were estimated (Table I). They are almost in agreement with the investigations of Page and Corcoran,[11] Oshima,[12] Smith[2] and others.[14-18]

Acute effect of hypotensive agents on renal hemodynamics in hypertensive patients

1. Hexamethonium bromide (C_6)

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The Effect of Hypotensive Agents on Renal Hemodynamics in Hypertensive Patients

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While most of clinicians attempt to reduce the elevated blood pressure in the treatment of hypertension, it remains still as an important problem to decide whether or not thus artificially lowering blood pressure is advantageous for the hypertensive patients.

Frey,[1] Smith[2] and others maintain that there exists vicious circle between hypertension and arteriolar changes or renal hemodynamic disorders, so that its interruption is recommendable as an excellent treatment of hypertension; according to them the artificial reduction of the blood pressure is favourable to the hypertensive patients. Sassa[3] suggests that in general the continued high blood pressure is harmful and its lowering with some treatments may remove the undesirable effects on the cardiovascular system, consequently the renal vascular damage being prevented to some extent. In this respect he agrees with Volhard's opinion.

Earlier Ito[4] observed in experimental hypertensive rabbits and patients of hypertension apparent decreases of glomerular filtration rate, tubular reabsorption and creatinine excretion when the blood pressure is lowered by giving nitrite salts. From these experimental data, Kato[5] inferred the elevated blood pressure may be a compensatory phenomenon to maintain the adquate blood supply to glomerulus and the sufficient urine volume, so that it is sometimes harmful to reduce the hypertension recklessly. However, he argues from another point of view, the adequate artificial lowering of the blood pressure may be favourable in hypertension, for it often leads to decrease of the renal vascular spasm and improvement of the renal blood flow. Indeed, in hypertension, there is spasm of afferent glomerular arterioles, causing a poor blood supply to glomerulus, consequently the continued hypertension leads to renal damage.

Recent advance in hypotensive agents brought a series of effective drugs, such as hexamethonium salts, veratrum alkaloids, 1-hydrazino-

phthalazine, etc. widely in practical use in hypertensive diseases, but their effects on cardiovascular and renal function are still under discussion, which is chiefly concentrated to the question of their therapeutic usefulness in the treatment of hypertension. Moyer[6], Oshima[7] and others inferred they should be used after measuring the change in the blood flow to various organs. Fishberg[8] and others[12][28] suggested that in the hypertension with severe arteriosclerosis in the important organs, such as heart, brain, kidney, etc., abrupt fall in blood pressure may lead to inadequate blood supply to these arteriosclerotic organs, consequently the artificial reduction of the blood pressure is disadvantageous, while in the absence of such arteriosclerotic complication, the patient will be favoured by lowering the blood pressure. In general, it seems that the effect of hypotensive agents is different in each case, according to the form as well as the severity of the hypertension, so their indication should be determined observing the reactions on each patient.

The purpose of the present investigation is to study the acute response of renal hemodynamics in hypertensive patients when the blood pressure is artificially lowered, using some hypotensive agents, because in hypertension the changes of renal hemodynamics play the most important role at every stage of the disease. An attempt is made to find the difference, if any, of the hemodynamic response between those agents, further between each stage of essential hypertension and renal hypertension, and to discuss on the above problem.

Experimental

Methods

Hexamethonium bromide, veratrum viride and 1-hydrazinophthalazine were injected intramuscularly or intravenously in 23 cases, including essential hypertension, malign hypertension and renal hypertension. The hemodynamic changes in kidney following the lowered blood pressure induced by these drugs were observed by measuring glomerular filtration rate (GFR) (mannitol-clearance), renal plasma flow (RPF) (*p*-aminohippuric acid-clearance), filtration fraction (FF) and renal vascular resistance (RVR), previously one or two times during 45 minutes before injection, and two or four times between 15 and 60 minutes after the injection of the hypotensive agents. The clearances were estimated by constant infusion technique.[9],[10] Estimations of *p*-aminohippuric acid (PAH), and mannitol were performed by the method of Page and Corcoran[11] with spectrophotometer after deproteinisation by Fujita-Iwatake's method.[11] GFR, RPF, renal blood flow (RBF), FF and RVR were calculated as follows:

GFR (cc/min.)=infused mannitol per min/concentration of mannitol in plasma,
RPF (cc/min.)=infused PAH per min/concentration of PAH in plasma,
RBF (cc/min.)=RPF/100—hematocrit reading,
FF=GFR/RPF,
RVR=MAP/RBF.

Blood pressure was determined at frequent intervals with Riva-Rocci's sphygmomanometer, and mean arterial pressure (MAP) was calculated as the third pulse pressure plus diastolic pressure. These values of clearance were corrected for standard body surface area 1.48 in sq. m. Throughout the experiment, the patients remained in the recumbent position to eliminate postural effects on blood pressure and on kidney function.

Classification of essential hypertension was made according to Schroeder[12]: I stage (mild benign hypertension), II stage (moderate benign hypertension), III stage (severe benign hypertension) and IV stage (malignant hypertension).

Results with Discussion

Renal hemodynamics in normotensive persons

Renal hemodynamic values of normotensive adults including 3 male and 2 female were estimated (Table I). Those values indicate an average with its reliable limit (α=0.05).

Renal hemodynamics in hypertensive patients

In 25 hypertensive subjects of several forms the renal hemodynamic values were estimated (Table I). They are almost in agreement with the investigations of Page and Corcoran,[11] Oshima,[12] Smith[2] and others.[14-18]

Acute effect of hypotensive agents on renal hemodynamics in hypertensive patients

1. Hexamethonium bromide (C_6)

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TABLE I

Renal Hemodynamic Values in Normotensive and Hypertensive Persons

The figures in parentheses show mean values.

	Cases	Ages	Blood pressure systolic / diastolic mm. Hg	Glomerular filtration rate cc/min	Renal plasma flow cc/min	Renal blood flow cc/min	Filtration fraction	Renal vascular resistance
Normotensive person	5	23-32	110-135 / 72- 85	111.4 ±15.9	578.0 ±105.0	1050.8 ±150.7	0.195 ±0.033	0.084 ±0.016
Essential hypertension II	9	46-69	158-220 / 62-122	96.2-140.6 (130.0)	447.3-764.0 (565.2)	732.1-1317.2 (939.0)	0.183-0.258 (0.229)	0.083-0.180 (0.139)
" " II～III	3	43-52	190-200 / 100-116	120.3-129.7 (124.9)	437.3-569.4 (517.6)	795.1-1035.0 (913.5)	0.220-0.285 (0.244)	0.127-0.170 (0.150)
" " III	1	63	210 / 115	94.5	382.7	673.8	0.247	0.212
" " III～IV	4	40-74	160-280 / 82-135	102.4-146.4 (128.1)	340.2-642.4 (441.4)	475.3-1207.5 (791.4)	0.228-0.419 (0.307)	0.102-0.252 (0.205)
" " IV	3	59-67	170-280 / 80-140	92.9-115.0 (101.6)	279.5-469.5 (359.0)	396.1-630.2 (478.7)	0.245-0.332 (0.291)	0.276-0.297 (0.289)
Chronic glomerulonephritis	4	29-56	155-220 / 90-140	96.4-139.3 (121.6)	248.7-335.3 (293.5)	326.0-545.2 (448.2)	0.306-0.507 (0.420)	0.264-0.343 (0.305)
Nephropathia gravidarum	1	37	180 / 100	105.3	659.5	1077.6	0.160	0.118

stage of III∼IV (malign hypertension), GFR (−14∼−22%, mean −17.7 %), RPF (0∼−14%, mean −8.7%), RBF (−1∼−15%, mean −10.0%) and FF (−6∼−13%, mean −9.3%) are depressed, on the contrary RVR increased (−4∼+14%, mean +7.0%). In chronic glomerulonephritis,

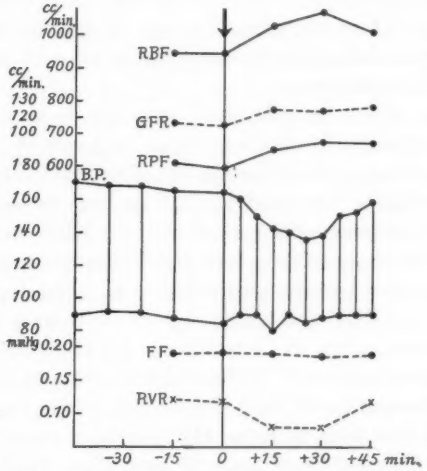

Fig. 1. S. K. aged 53, benign hypertension. ↓....37.5 mg. C_6 i.m.

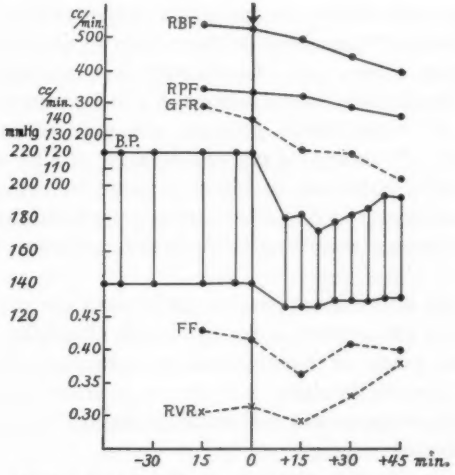

Fig. 2. S. Z. aged 56, glomerulonephritis chronica. ↓....25 mg. C_6 i.m.

the changes are as follows: GFR +4~−27%, mean −14.4%, RPF +30 ~−23%, mean +1.4%, RBF +30~−25%, mean +0.6%, FF +5~−38%, mean −12.9% and RVR +22~−27%, mean −3.6%; thus, they are similar to those in malignant hypertension, but milder. (Fig. 2)

This series of estimations in hypertensive disease show clearly that there is a definite difference in the changes of renal hemodynamic values between the stage II or the stage II~III of essential hypertension, and malign hypertension belonging to the stage of III~IV or chronic glomerulonephritis with hypertension.

On the acute renal hemodynamic response to the administration of C_6, Ullman and Diengott[19] observed in the majority of hypertension without apparent renal failure increased RBF, decreased GFR, FF and RVR. Also, Mills and Moyer[20] remarked that in hypertension with no renal damage, after intravenous administration of C_6 RBF increased and RVR decreased, while in those with severe renal insufficiency RBF, GFR and RVR tended together toward depression. Kirkendal and Culbertson[21] found an increase in RBF and no change in GFR in 3 cases of moderate essential hypertension after the intravenous injection of 20 to 160 mg. of C_6. Oshima and co-workers[22] confirmed after the injection of C_6 increase in RBF and reduction in FF and RVR, when the reduction of systolic blood pressure is less than 30 mm. Hg; on the contrary depression in them when it is lowered more than 30 mm. Hg. According to Mackinnon[23], the intravenous injection of varying amounts of pentamethonium (10 to 100 mg.) is followed by reduced GFR and RBF, and increased RVR in patients with benign or malignant hypertension. Mills and co-workers[24] and Blainey[25] observed on the whole similar responses to C_6 in hypertension. Also Moyer and co-workers[26] found a significant decrease of RBF by intramuscularly administering C_6 in hypertension, but it was the case when the mean blood pressure was lowered more than 50%. Grob and co-workers[27] estimated prompt decrease of RBF and GFR under on an average 25% reduction in blood pressure in benign and in early phase of malignant hypertension after intravenous administration of C_6, the decrease of RBF varying according to the degree and rate of lowered blood pressure.

In most of the above mentioned investigations the rate of blood pressure reduction was too servere to be applicable clinically. In the present investigation, the grade of blood pressure reduction following the administration of hexamethonium was on an average 14.6% in essential hypertension without apparent renal findings and 6.2% with renal damage or glomerulonephritis.

In the previous papers treating on the renal hemodynamic response to hexamethonium, discussion has been chiefly made on the difference

in the range of blood pressure reduction, neglecting the stage or form of hypertensions. In the present investigation on the renal hemodynamic response to C_6, as above mentioned, an opposite relation on the change of RBF and RVR has been observed between the essential hypertension without apparent renal damage and the malignant hypertension or hypertensive chronic glomerulonephritis. It may be suggested that in hypertensive subjects whose kidney function is not yet apparently damaged by hypertensive vascular changes, hexamethonium acts beneficially upon them during the lowered blood pressure, forming an indication for therapeutic use, while in the hypertension with renal insufficiency it must be used only in selected cases under careful caution for its undesirable reactions.[29] It should be avoided to lower the elevated blood pressure rapidly and excessively with hexamethonium, and at the first the form and the stage of the hypertensive disease should be exactly diagnosed before its administration. Grob and Langford[27] pointed out that after the administration of hexamethonium the blood non-protein-nitrogen indicates no change in benign hypertension, but an increase in malignant hypertension.

2. Veratrum alkaloids

Observation on renal hemodynamics were made on 8 hypertensives giving 0.6 to 0.8 mg. of veratrum viride (Trapon) intramuscularly, only in 1 case with intravenous administration of 2 mg. Of 8 hypertensives 3 (syst. 196–206, diast. 60–112 mm. Hg) were at II stage, 1 (syst. 190, diast. 116 mm. Hg) at II∼III stage, 3 (syst. 190–280, diast. 90–150 mm. Hg) at III∼IV stage and the remaining one (syst. 180, diast. 100 mm. Hg) was nephropathia gravidarum with hypertension. Through the estimations, it has been ascertained that in essential hypertension at the stages of II and III, GFR is not altered significantly or decreased slightly (+2∼−10%, mean −4.5%), RPF (−3∼+40%, mean +15.8%) and RBF (+2∼+35%, mean+16.1%) increased apparently, FF (+5∼−17%, mean −7.3%) and RVR (−12∼−40%, mean −23.3%) decreased, while at the stage of III∼IV (malign hypertension), GFR (+6∼−26%, mean −16.6%), RPF (−2∼−21%, mean −8.8%), RBF (−2∼−21%, mean −9.2%), FF (0∼−14%, mean −8.4%) and RVR (−4∼−29%, mean −15.6%) all together decreased. (Fig. 3) Changes in nephropathia gravidarum were similar to those in malignant hypertension, but was milder: GFR−3%, RPF −2%, RBF±0%, FF −2% and RVR −4%. Thus, also with veratrum viride similar difference in the renal hemodynamic responses has been ascertained.

Hoobler and co-workers[30] report, in hypertensive patients after administration of protoveratrine GFR decreases moderately, while RBF remains constant or increases with consequent reduction in FF, but there is no mention concerning the form or stage of the observed hypertension.

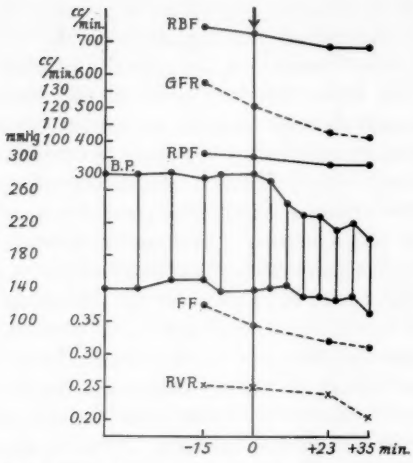

Fig. 3. T. K. aged 54, malign hypertension. ↓....0.6 mg. veratrum alkaloid (Trapon) i. m.

Mitsui and co-workers[31] remarked in malign hypertension after the intravenous injection of Trapon (0.8 mg. veriloid) reduction in GFR and RBF; Freis and co-workers[32] found in 3 cases of chronic glomerulonephritis no change or depression of RPF and decrease of FF after intramuscular administration of veratrone or vertavis. Iwahashi[33] has ascertained in the toxemia of pregnancy depression of renal function after apparent reduction of the blood pressure due to veratrum viride. According to Wilkins[34] in hypertension, RBF shows an initial decrease with lowered blood pressure following the veratrum injection, but it returns to the initial value when the pressure becomes stabilized at lower levels. Freis[32] reports 7 cases of essential hypertension, in which after intramuscularly administered veratrone, RPF after initial fall returns to approximate control value, despite a continued hypotensive response, and 5 cases, in which RPF exceeds the initial levels to some extent, while GFR shows a slight reduction and FF no change or decrease. Those findings seem to be manifest especially in intensively induced hypotension. Meilman[35] has found in 8 hypertensive patients reduction in GFR and RPF after intravenous protoveratrine injection, while with intramuscular administration, the renal hemodynamic changes are similar to those observed by Freis[22], RVR is reduced in all.

In above mentioned reports, in which decrease of RBF with veratrum has been observed, there is no description concerning the form of hypertension, and the grade of lowered blood pressure seems too severe. Gold-

man and Frierson[36], who observed the effect of intravenous veriloid on renal function in 19 hypertensives, found in all cases with the systolic pressure of 185 mm. Hg or more, decreased RPF and increased FF or both reactions, while in cases with lower initial pressure GFR increased slightly and FF fell remarkably. No considerations were given concerning the renal function. From the results of the present study it may be suggested that in benign hypertension a moderate reduction of blood pressure induced with veratrum alkaloids is beneficial to the renal function, while in malign hypertension or renal hypertension it is followed by decrease of renal blood flow despite of reduced FF and RVR, so that this agent should be applied with serious considerations in the latter.

3. 1-Hydrazinophthalazine (Apresoline)

9 hypertensives got 10 to 20 mg. of apresoline intramuscularly. Of those 9 hypertensives were 4 cases (syst. 182–220, diast. 94–122 mm. Hg) at II stage, 1 case (syst. 195, diast. 105 mm. Hg) at II~III stage, 1 case (syst. 198, diast 110 mm. Hg) at III stage, 2 cases (syst. 190–280, diast. 84–140 mm. Hg) at IV stage and 1 case (syst. 185, diast. 126 mm. Hg) with hypertensive chronic glomerulonephritis. Estimations showed in essential hypertension at the stage of II and III, GFR (+5~−22%, mean −8.9%) decreased, RPF (−15~+28%, mean +11.8%) and RBF (−15~+31%, mean +14.5%) increased, FF (+3~−30%, mean −17.3%) and RVR (−9~−37%, mean −19.7%) decreased (Fig. 4), while in malignant hypertension GFR (0~−39%, mean −20.2%), RPF (−15~−35%, mean

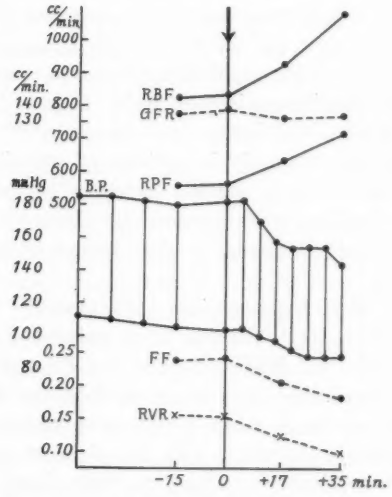

Fig. 4. T. O. aged 61, benign hypertension. ↓ .... 12 mg. apresoline i.m.

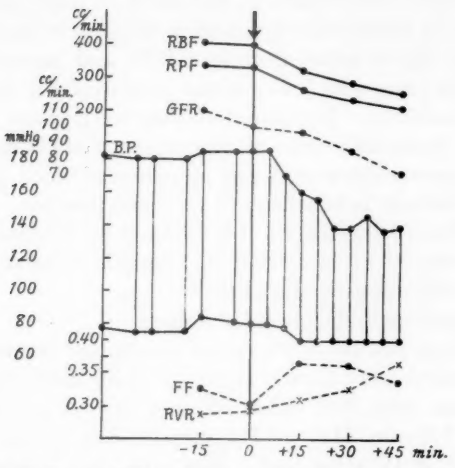

Fig. 5. F. K. aged 65, malign hypertension. ↓....20 mg. apresoline i.m.

24.4%) and RBF (−15∼−36%, mean −24.6%) decreased, FF (+23∼−19%, mean +6.0%) and RVR (+4∼+24%, mean +12.8%) increased (Fig. 5). In chronic glomerulonephritis moderate reduction of GFR (−15%), FF (−10%) and RVR (−17%), slight decrease of RPF (−6%) and RBF (−1%) were observed; the changes were milder than in malignant hypertension. Thus, it has been confirmed that also with apresoline the renal hemodynamic changes are similar as with C_6 and veratrum.

With respect to the acute renal hemodynamic response to apresoline after its intravenous administration, Reubi[37] observed increase of RBF and reduction of RVR, Wilkinson and co-workers[38] augmented RBF without rise of GFR, Mackinnon[23] in patients of essential hypertension increase of RBF and lessening of RVR, thereby GFR showing no definite change. The same findings were reported by Gross,[39] Moyer,[40] Freis,[41] Riven[42] and others, and are similar to the changes in benign hypertension in the present paper.

However, in malign hypertension (Mackinnon)[23] or in azotemia (Hafkenschill and Lindauer)[43], it has been pointed out that after intramuscular apresoline injection RBF increased and FF and RVR diminished. They maintain that apresoline gives rise to an increase of RBF in any form of hypertension, different from other hypotensive agents.[2][12][44] Some[11][28] indicate that apresoline is an effective agent in the treatment of malignant or renal hypertension. On the contrary to their observations, the present investigations have confirmed that in malign hypertension after the ad-

ministration of apresoline RBF is reduced and RVR increased, while in chronic glomerulonephritis with hypertension RBF has shown no definite change, thereby RVR being reduced. It is of clinical interest that these findings were obtained when the blood pressure was moderately lowered. Kummer and von La Rosée[45] infer that in chronic hypertensive glomerulonephritis RPF and GFR are decreased and FF increased with hydrazinophthalazine derivate. Others[12][46-47] maintain that apresoline is little effective in the hypertension with renal damage.

From the present results it may be suggested that apresoline should be used in the hypertension without apparent renal changes, and be applied only under a serious consideration to the malign hypertension or renal hypertension. Overdosage of the drug, leading to sudden and severe reduction in blood pressure, should be avoided, even though in benign hypertension.[38] In one case of benign hypertension, in which the blood pressure has been reduced from 198/110 to 136/80 mm. Hg, RBF decreased from 690 to 586 cc./min.

Conclusion

The present investigation was performed to study the acute renal hemodynamic responses following the injection of hexamethonium bromide, veratrum alkaloids or 1-hydrazinophthalazine (apresoline) in hypertension of different origins. In the essential hypertension with no or little renal change (I to III stage according to Schroeder's classification)[12], there are on the whole an increase in renal blood flow and reduction in filtration fraction and renal vascular resistance, when the blood pressure is reduced. Any definite difference in the effects between each drug could not particularly be seen, except for glomerular filtration rate, which shows a tendency of slight increase after hexamethonium administration and no change or a little decrease after veratrum or apresoline. But in malign hypertension with renal insufficiency, common to all those three hypotensive agents, glomerular filtration rate, renal blood flow and filtration fraction are reduced, while renal vascular resistance is increased after hexamethonium or apresoline, but decreased after veratrum. In chronic glomerulonephritis and nephropathia gravidarum with hypertension, the renal hemodynamic responses to those agents are similar to those in malign hypertension, but less pronounced.

As in the essential hypertension with no or very little renal damage those drugs cause a rise in renal blood flow and a fall in renal vascular resistance during the lowered blood pressure, thus act on the renal function favourably, it seems desirable clinically to apply them in such cases. On the contrary in malign hypertension and to some extent also in hypertensive chronic glomerulonephritis, an opposite effect has been confirmed, so that

it may be inferred that those agents might lead to impair the renal function, and should be used under careful caution and limited conditions.

Those findings suggest that the hypotensive agents should be applied always following the diagnostic determination of the form and stage of hypertension, especially after exact investigation of the renal function. Next, it is important to determine the advantageous limit for lowering the blood pressure; but it must be born in mind that the hypotensive response to drugs vary according to the arteriolar change and the degree of vascular resistance, thus offering a very complicated problem. As a rule, it should be abstained from lowering the elevated blood pressure too rapid and too severe. The reduction is desirable to be restrained within approximately thirty percent in mean arterial pressure even in hypertension without renal failure; this is especially the case in more advanced hypertension.

The chief purpose of the treatment with hypotensive agents seems to consist in the prevention of the arteriosclerotic process by relaxing the spasm of arterioles. It is the subject of further research to study the chronic effects following the continuous administration of those agents. The changes in renal hemodynamics as one of such effects are now under investigation.

Summary

1. The glomerular filtration rate, renal plasma flow, renal blood flow, filtration fraction and calculated renal vascular resistance were estimated in 5 normotensive, 20 benign and malignant hypertensive and 5 renal hypertensive subjects.

2. The acute effect on renal hemodynamics of hexamethonium, veratrum alkaloids or apresoline, parenterally administered, were investigated in 28 hypertensive patients including 23 benign and malignant hypertension and 5 renal hypertension.

In benign hypertension, there were in general an increase in renal plasma flow, renal blood flow, and a reduction in filtration fraction or renal vascular resistance following the administration of each hypotensive agent, but glomerular filtration rate shows a slight increase after hexamethonium and no change or slight decrease after veratrum or apresoline.

In malignant hypertension and in renal hypertension, though in the latter to a less degree, the glomerular filtration rate, renal plasma flow, renal blood flow and filtration fraction are reduced, common to all three drugs, but renal vascular resistance shows a depression after veratrum and an increase after hexamethonium or apresoline. Differences between the responses to each agent are not significant.

3. It is discussed whether or not artificially lowering blood pressure

using hypotensive agents is advantageous in hypertensive patients; further, the indication for the blood pressure reduction is argued.

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Studies on the Specific Antigens of Human Gastric Cancer. I

By

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Hirszfeld, Witebsky, Lehmann-Facius, and others[1)-4)] found that there exist antigens specific to cancer in the alchohol extract of human cancers and some of them bear close relations to embryonal tissue. Essentially the same results were obtained also by Sakuragi[9)], Furusawa[14)], Matsuo[15)], and others in our country. Lehmann-Facius[13)] asserts that the so-called phospholipid fraction of the alchohol extract, a fraction soluble in benzene and insoluble in acetone and soluble in petroleum ether, is responsible for this specific antigenicity. Further information as to the nature of specific antigens of cancer, however, has not been obtained yet. Thus, I have undertaken a series of studies with human gastric cancer to clear up this point. The work recorded in the present paper is the first of the series, in which the presence of specific antigens in the alcohol extract of human gastric cancer has been demonstrated.

Experimental

Material and Method

Essentially, the experiments consisted of preparing rabbit sera against alcohol extracts of human gastric cancer and normal gastric mucosa, and performing complement fixation tests of these two antisera, aided by absorption tests. In carrying out the serological test I received generous guidance of Prof. Murakami (director) and members of the legomedical Institute of our university.

1) Preparation of the alcohol extracts of a tissue

The materials used were fresh specimens of (1) the gastric cancer obtained from the excised stomachs of patients with gastric cancer in our clinic, (2) the normal gastric mucosa obtained from the excised stomachs of patients with gastric or duodenal ulcer, and (3) the villous tissue of placenta obtained in the gynecological clinic immediately after birth. A specimen of a tissue was cut into fine pieces and well washed in water

to remove blood. Excess water being removed with filter paper, about 10 times as much 95 per cent alcohol was added, and the mixture was left at room temperature for about 1 month. The resulting alcohol extract was filtered and evaporated under reduced pressure at 37 to 40°C., and the condensed material was kept in a desiccator until used.

2) Preparation of antigens for immunization

According to Witebsky[7], Hirszfeld[8], Sakuragi[9], and others, when a rabbit is immunized with cancerous tissue from a patient of blood group A, an antibody specific to group A, which is liable to be confounded with the cancer-specific antibody, will be produced. Therefore, the alcohol extracts of the two tissues obtained only from patients of blood group O or B were used as immune antigens. The activator was prepared after Landsteiner[5], and the adjuvant by referring to Freund's method[6] as follows: An amount of 2.0 g. of an alcohol extract was weighed into a glass mortar, and well spread, to which was added little by little 50 cc. of 5 per cent solution of swine serum in physiological saline (containing 0.5 per cent phenol) to obtain a homogenous milky suspension by thorough mixing. The suspension was slowly added to an agitator containing 2.50 cc. of dehydrated lanolin, which had previously been dissolved at 30°C., to form a homogeneous suspension by constant stirring. On the other hand, 50.0 mg. of heat-killed human tubercle bacilli (Aoyama B strain) was weighed into an agate mortar and well ground, to which was added 2.50 cc. of paraffin oil to obtain a homogeneous suspension by thorough mixing. This suspension was dropped, while stirring, into the above-mentioned milky suspension in the agitator. Then, 75.0 cc. of paraffin oil was further added to the resulting mixture, and kept stirring for 3 or 4 hours to obtain a creamy suspension. The suspension was kept in a tightly stoppered brown bottle in an ice chamber until needed as an immune antigen.

3) Immunization

As it has been pointed out that the production of group-specific antibodies in a rabbit is related with its serum group and saliva group (Aoki[10], Yokoyama[11], Murakami[12], and others), I used in the present study 20 healthy rabbits (weighing about 2.5 kg.) of serum group O′ or β' and saliva group A (+) to prevent the production of group-specific antibodies.

Each rabbit received 9 intramuscular injections of 5.0 cc. of an antigenic preparation in the bottocks (on the right and the left side alternately) at intervals of twice a week. On the 7th day after the last injection a preliminary titration was made; and when the titer was not high enough, a second series of injections was administered in the same manner as before, beginning on the 14th day after the last injection of the first series. When the titer was found to have become high enough, the rabbit was

bled from the carotid artery on the 7th day after the last injection. The serum was separated, inactivated for 30 minutes at 56°C., preserved with phenol (0.5 per cent), placed in a brown bottle, and stored in an ice chamber. The rabbit serum thus obtained against the alcohol extract of a human tissue will hereafter be referred to simply as the serum against that tissue.

4) Complement fixation tests

Complement fixation tests were performed with varying dilutions of antigen (antiserum being used at a fixed concentration). First, a 0.5 per cent solution of an antigenic preparation in alcohol was prepared, which will be referred to as the stock antigen. Antigen for use with complement fixation tests were made up by various dilutions of this stock antigen. These dilutions were made up by adding while stirring given amounts of the stock antigen to given amounts of physiological saline.

The results of complement fixation tests were read as follows: ⧺, complete hemolysis; ⧺, ⧺, ⧺, +, ±, ∓, incomplete hemolysis in 6 grades; −, complete non-hemolysis.

5) Absorption tests

The necessary and sufficient amount of the alcohol extract of a tissue, which had been found by preliminary tests, was accurately taken, added always to 1 cc. of an antiserum to be absorbed, well mixed with a glass rod to give a milky suspension, left for 1 hour, and kept in an ice chamber for 3 hours. The antiserum thus absorbed with the tissue was filtered through asbestos; in order to obtain uniform results care was taken to make the filling of asbestos always of the same extent.

Result

Complement fixation tests of the sera against human gastric cancer and human normal gastric mucosa (hereafter referred to simply as gastric cancer and gastric mucosa, respectively) with alcohol extracts of gastric cancer, normal gastric mucosa, and placenta were performed with the results that with the serum against gastric cancer, all the three extracts showed fairly intense reactions (Table IA), where as the extract of placenta did not react with the serum against normal gastric mucosa, different from the other two extracts (Table IB). This indicates that some antigens were common to both tissues: gastric cancer and placenta.

When the serum against gastric cancer was absorbed with 10 mg. of the placenta extract per 1 cc., the antiserum reacted both with the alcohol extract of gastric cancer and with the extract of normal gastric mucosa, as shown in the upper left corner (surrounded by heavy lines) of the main part of Table IA. However, when 8 mg. of the extract of normal gastric mucosa per 1 cc. of the serum was used for absorption,

TABLE IA

Antiserum : Rabbit serum against alcohol extract of human gastric cancer, diluted 1 :30.
Antigen : Alcohol extract of each tissue, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen Alcohol extract of	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
First: 10 mg. alcohol extract of human placenta Second: 8 mg. alcohol extract of normal human gastric mucosa	Human gastric cancer	−	−	−	−	−	−	−	±	++	+++	+++
	Normal human gastric mucosa	−	−	−	−	−	−	+	++	+++	+++	+++
	Human placenta	−	−	−	−	−	++	++	+++	+++	+++	+++
	Control antiserum											+++

TABLE IB

Antiserum : Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1 :30.
Antigen : Alcohol extract of each tissue, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen Alcohol extract of	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
40 mg. alcohol extract of human gastric cancer	Normal human gastric mucosa	−	−	−	−	−	−	−	−	++	+++	+++
	Human gastric cancer	−	−	−	−	±	++	+++	+++	+++	+++	+++
	Human placenta	∓	+	++	+++	+++	+++	+++	+++	+++	+++	+++
	Control antiserum											+++

the antiserum reacted only with the extract of gastric cancer, as shown in the upper left corner (surrounded by broken lines) of Table IA.

When the serum against normal gastric mucosa was absorbed with 40 mg. of the gastric cancer extract per 1 cc., the complement fixation tests yielded the results that only the alcohol extract of normal gastric mucosa still reacted, as shown in the upper left corner (surrounded by heavy lines) of Table IB. From these it is clear that in the alcohol extract of gastric cancer there were present antigens partly different from those cotained in the extrakt of normal gastric mucosa, i.e., antigens specific to the extract of gastric cancer.

SUMMARY

The presence of specific antigens in the alcohol extract of human gastric cancer has been demonstrated.

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Studies on the Specific Antigens of Human Gastric Cancer. II

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In the preceeding paper of this series, the presence of specific antigens in the alcohol extract of human gastric cancer was demonstrated. Based upon this fact, attempts have been made to trace the specific antigenicity with carefully separated fractions of the alcohol extract, with fairly satisfactory results to be described in the following.

Experimental

Material and Method

Essentially, the experiments consisted of preparing rabbit sera against alcohol extracts of human gastric cancer and normal gastric mucosa, and performing complement fixation tests of these two antisera, aided by absorption tests, with various fractions of the alcohol extracts.

The procedures for (1) preparation of the alcohol extract of a tissue, (2) preparation of antigens for immunization, (3) immunization, (4) complement fixation tests, and (5) absorption tests were exactly the same as those described in the preceeding paper.

(6) Fractionation of the alcohol extract of a tissue.

In carrying out the fractionation I received generous guidance of Prof. H. Masamune (director) and members of the Medicochemical Institute of our University.

The specific antigens in the alcohol extract of human gastric cancer (hereafter referred to simply as gastric cancer similarly, human gastric mucosa as gastric mucosa) were traced as follows: After the presence of antigens specific to gastric cancer was confirmed by complement fixation tests with the alcohol extracts of the three tissues and the two antisera, the alcohol extracts of gastric cancer and normal gastric mucosa were fractionated with organic solvents, as shown schematically in Table I. By referring to the results of the complement fixation test with each fraction as antigen, the fractionation was made finer and finer until the fraction

TABLE I

Fractionation of Alcohol Extract of Human Gastric Cancer

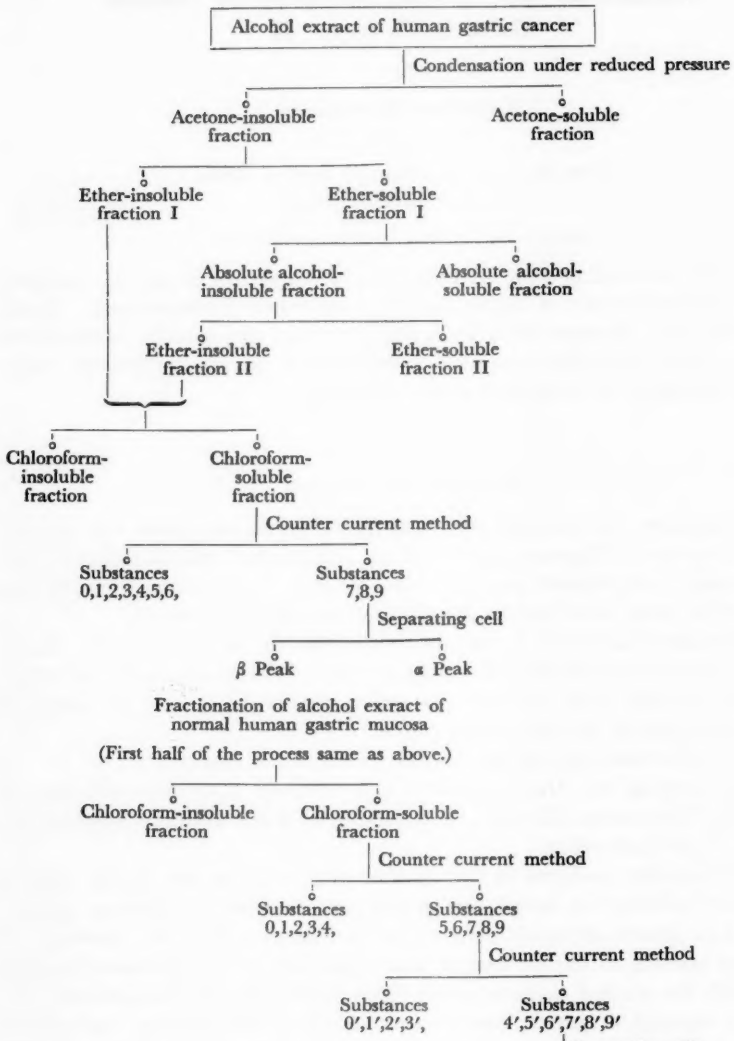

TABLE IIA

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1:150. Antigen: Acetone-sluble or -insoluble fraction of human gastric cancer, doubling dilutions of 0.5% stock antigen, starting from 1:20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
8 mg. alcohol extract of normal human gastric mucosa	Acetone-soluble fraction of human gastric cancer	−	−	−	−	−	−	+	++	###	###	###
	Acetone-insoluble fraction of human gastric cancer	−	−	−	−	−	−	−	±	++	###	###
	Control antiserum											###

TABLE IIB

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1:150. Antigen: Acetone-soluble or -insoluble fraction of normal human gastric mucosa, doubling dilutions of 0.5% stock antigen, starting from 1:20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
20 mg. alcohol extract of human gastric cancer	Acetone-soluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	+	++	###	###
	Acetone-insoluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	−	−	±	###
	Control antiserum											###

responsible for the specific antigenicity of gastric cancer could give a single peak in an electrophoretic pattern when submitted to electrophoresis by the Tiselius method.

Result

1) Tests with the acetone fractions (Table II A, B)

The alcohol extracts of gastric cancer and normal gastric mucosa were treated with dehydrated acetone to separate acetone-soluble and -insoluble fractions. A 0.5 per cent solution of each fraction in alcohol was prepared, from which various dilutions were made from complement fixation tests. The results of the tests with these dilutions and the two antisera are given in Table II (A, B). It will be seen that in the tests with the serum against

TABLE IIIA

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1 :150. Antigen: I. ether-soluble or -insoluble fraction of human gastric cancer, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
8 mg. alcohol extract of normal human gastric mucosa	I. ether-soluble fraction of human gastric cancer	−	−	−	−	−	±	+	+	++	++	++
	I. ether-insoluble fraction of human gastric cancer	−	−	−	−	−	−	−	±	+	++	++
	Control antiserum											++

TABLE IIIB

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1 :150. Antigen: I. ether-soluble or -insoluble fraction of normal human gastric mucosa, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
20 mg. alcohol extract of human gastric cancer	I. ether-soluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	−	++	++	++
	I. ether-insoluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	−	−	++	++
	Control antiserum											++

gastric cancer the acetone-soluble and -insoluble fractions reacted with the unabsorbed serum to nearly the same extent, but when absorbed with the extract of normal gastric mucosa, the antiserum reacted only with the acetone-insoluble fraction, as shown in the surrounded area of Table II A. Similarly, when absorbed, the serum against normal gastric mucosa reacted only with the acetone-insoluble fraction (Table II B, surrounded area).

All these results indicate that the acetone-soluble fractions of both alcohol extracts of gastric cancer and normal gastric mucosa possessed common antigens, while the acetone-insoluble fractions of both extracts had no common ones.

2) Tests with ether fractions I (Table III A, B)

The aceton-insoluble fractions of both gastric cancer and normal gastric mucosa were separated with dehydrated ether into ether soluble

TABLE IVA

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1 :150. Antigen: Absolute alcohol-soluble or -insoluble fraction of human gastric cancer, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
8 mg. alcohol extract of normal human gastric mucosa	Absolute alcohol-soluble fraction of human gastric cancer	−	−	+	++	++	++	++	++	++	++	++
	Absolute alcohol-insoluble fraction of human gastric cancer	−	−	−	−	−	+	++	++	++	++	++
	Control antiserum											++

TABLE IVB

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1 :150. Antigen: Absolute alcohol-soluble or -insoluble fraction of normal human gastric mucosa, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
16 mg. alcohol extract of human gastric cancer	Absolute alcohol-soluble fraction of normal human gastric mucosa	−	−	−	±	+	++	++	++	++	++	++
	Absolute alcohol-insoluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	+	+	++	++
	Control antiserum											++

fraction I and -insoluble fraction I. The complement fixation tests of the two antisera with both fractions yielded the results that with the unabsorbed antisera no significant differences were observed between the reactions of both fractions, whereas with the absorbed antisera with ether-insoluble fraction I reacted more intensely (Table III A, B surrounded areas). It was necessary, however, to separate specific antigens in ether-soluble fraction I, for this fraction also reacted to some extent.

3) Tests with the absolute alcohol fractions (Table IV A, B)

Ether-soluble fractions I of gastric cancer and normal gastric mucosa were separated with absolute alcohol into alcohol -soluble and -insoluble fractions. Though the unabsorbed antiserum reacted with both fractions,

the absorbed antiserum reacted only with the alcohol-insoluble fraction (Table IV A, B, surrounded areas). This is indicative of the transferrence of the specific antigenicity to the alcohol-insoluble fraction.

4) Tests with ether fraction II (Table V A, B)

TABLE VA

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1:150. Antigen: II. ether-soluble or -insoluble fraction of human gastric cancer, doubling dilutions of 0.5% stock antigen, starting from 1:20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
8 mg. alcohol extract of normal human gastric mucosa	II. ether-soluble fraction of human gastric cancer	−	−	−	++	###	###	###	###	###	###	###
	II. ether-insoluble fraction of human gastric cancer	−	−	−	−	−	−	±	++	###	###	###
	Control antiserum											###

TABLE VB

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1:150. Antigen: II. ether-soluble or -insoluble fraction of normal human gastric mucosa, doubling dilutions of 0.5% stock antigen, starting from 1:20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
20 mg. alcohol extract of human gastric cancer	II. ether-soluble fraction of normal human gastric mucosa	−	−	++	###	###	###	###	###	###	###	###
	II. ether-insoluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	+	++	###	###
	Control antiserum											###

The before-mentioned ether-insoluble fraction I and the alcohol-insoluble fraction, which had both been found to show antigenic differences between the extracts of gastric cancer and normal gastric mucosa, were mixed, treated again with dehydrated ether, and separated into ether-soluble fraction II and -insoluble fraction II. Complement fixation tests with these two fractions gave the results that even with the unabsorbed serum the reaction of ether-insoluble fraction II was stronger than that of ether-

soluble fraction II, while with the absorbed serum only ether-insoluble fraction II reacted (Table V A, B surrounded areas).

5) Tests with the chloroform fractions (Table VI A, B)

TABLE VIA

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1 :150. Antigen: Chloroform-soluble or -insoluble fraction of human gastric cancer, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
8 mg. alcohol extract of normal human gastric mucosa	Chloroform-soluble fraction of human gastric cancer	−	−	−	−	−	−	−	++	++	++	++
	Chloroform-insoluble fraction of human gastric cancer	−	−	−	−	+	++	++	++	++	++	++
	Control antiserum											++

TABLE VIB

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa diluted 1 :150. Antigen: Chloroform-soluble or -insoluble fraction of normal human gastric mucosa, doubling dilutions of 0.5% stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
20 mg. alcohol extract of human gastric cancer	Chloroform-soluble fraction of normal human gastric mucosa	−	−	−	−	−	−	−	−	++	++	++
	Chloroform-insoluble fraction of normal human gastric mucosa	−	−	−	−	−	−	+	++	++	++	++
	Control antiserum											++

In order to purify the specific antigens further, ether-insoluble fraction II was separated with chloroform into chloroform-soluble and -insoluble fraction. From tests with these fractions it was found that after absorption the antiserum reacted with the chloroform-soluble fraction much more intensely than with the chloroform-insoluble one (Table VI A, B, surrounded areas). These chloroform-soluble fractions of gastric cancer and normal gastric mucosa gave electrophoretic patterns with 4 peaks (Fig. I A, B), being suggestive of the presence of at least 4 kinds of substances there.

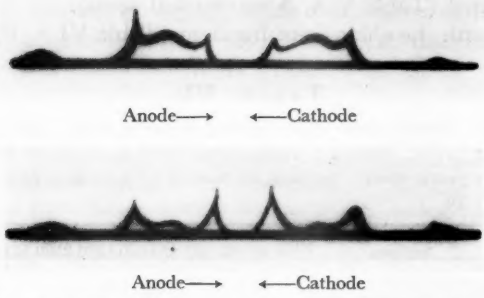

Fig. 1. Electrophoretic patterns of chloroform-soluble fractions of human gastric cancer (A) and normal human gastric mucosa (B), 1% solutions, carbonate buffer, pH 9.62.

6) Tests with the counter current fractions (Tables VII, VIII, IX)

In order to purify the specific antigens of gastric cancer further the counter current method was applied as follows: A mixture of benzene, methanol, toluene, and water (4 :4 :1 :1) was used as solvent and the chloroform-soluble fractions of gastric cancer and normal gastric mucosa were each separated into 10 fractions (substances 0 to 9). The results of complement fixation tests with these fractions are given in Tables VII and VIII. It will be seen that the specific antigens had transferred to substances 7 to 9 in the case of gastric cancer, and to substances 5 to 9 in the case of normal gastric mucosa. As the yields of substances 5 to 9 were comparatively large in the case of normal gastric mucosa, the counter current method was repeated using the same solvent mixture, and substances 4′ to 9′ were obtained (Table IX).

7) Tests with the fractions obtained with the separating cell (Table X A, B)

The mixture of substances 7 to 9 of gastric cancer and the mixture of substances 4′ to 9′ of normal gastric mucosa were submitted to electrophoresis, with the results that the substances responsible for the specific antigenicity were represented by two peaks, α and β in the former cases, and α' and β' in the latter (Fig. 2 A, B). The substances represented by these two peaks (substances α, β and substances α', β') were then separated by means of the separating cell. Complement fixation tests with these substances gave the results that little differences in their intensity of reaction were found between substances α and β, or between α' and β' (Table X A, B), showing a close resemblance in nature between substances α and β or between substances α' and β'. Further it will be seen that even if the serum against gastric cancer was absorbed with substances 4′ to 9′ of

TABLE VII

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1 :150. Antigen: One of substances 0 to 9 of human gastric cancer, doubling dilutions of stock antigen starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
15 mg. alcohol extract of normal human gastric mucosa	Substance 9 of human gastric cancer	−	−	−	+	++	+++	+++	+++	+++	+++	+++
	8	−	−	−	−	±	+	++	+++	+++	+++	+++
	7	−	−	−	−	−	+	++	+++	+++	+++	+++
	6	−	−	−	−	−	−	++	++	+++	+++	+++
	5	−	−	−	−	−	−	++	+++	+++	+++	+++
	4	−	−	−	−	−	−	++	+++	+++	+++	+++
	3	−	−	−	−	−	++	++	+++	+++	+++	+++
	2	−	−	−	−	+	++	+++	+++	+++	+++	+++
	1	−	−	+	++	+++	+++	+++	+++	+++	+++	+++
	0	−	−	−	−	+	++	+++	+++	+++	+++	+++
	Control antiserum											+++

TABLE VIII

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1 :150. Antigen: One of substances 0 to 9 of normal human gastric mucosa, doubling dilutions of stock antigen starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
25 mg. alcohol extract of human gastric cancer	Substance 9 of normal human gastric mucosa	−	−	−	−	−	−	−	+	++	++	+++
	8	−	−	−	−	−	−	−	++	+++	+++	+++
	7	−	−	−	−	−	−	−	−	++	+++	+++
	6	−	−	−	−	−	−	−	−	±	++	+++
	5	−	−	−	−	−	−	−	±	++	+++	+++
	4	−	−	−	−	±	++	+++	+++	+++	+++	+++
	3	−	−	−	−	∓	++	+++	+++	+++	+++	+++
	2	−	−	−	−	±	++	+++	+++	+++	+++	+++
	1	−	−	−	−	−	++	++	+++	+++	+++	+++
	0	−	−	+	++	+++	+++	+++	+++	+++	+++	+++
	Control antiserum											+++

normal gastric mucosa, the reaction was the same as that with unabsorbed serum (Table X A, surrounded area). Similarly, the reaction of the serum

Table IX

Antiserum: Rabbit serum against alcohol extract of normal human gastric mucosa, diluted 1 :150. Antigen: One of substances 0′ to 9′ of normal human gastric mucosa, doubling dilutions of stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
25 mg. alcohol extract of human gastric cancer	Substance 9′ of normal human gastric mucosa	−	−	−	+	++	###	###	###	###	###	###
	8′	−	−	−	−	−	−	++	++	###	###	###
	7′	−	−	−	−	−	−	−	+	++	###	###
	6′	−	−	−	−	−	−	+	++	###	###	###
	5′	−	−	−	−	+	++	###	###	###	###	###
	4′	−	−	−	−	++	###	###	###	###	###	###
	3′	−	−	−	+	++	++	###	###	###	###	###
	2′	−	−	++	###	###	###	###	###	###	###	###
	1′	−	+	++	###	###	###	###	###	###	###	###
	0′	±	++	###	###	###	###	###	###	###	###	###
	Control antiserum											###

against normal gastric mucosa after being absorbed with substances 7 to 9 of gastric cancer was the same as that of the unabsorbed serum (Table X B, surrounded area).

8) Qualitative analyses

As substance α of gastric cancer and substance α' of normal gastric mucosa presented each an electrophoretic pattern bearing a single peak (Fig. 3 A, B), each substance should be regarded as a single substance, not a mixture of two or more substances. Qualitative analyses of these substances gave the results: phosphorus test, ++; Molish's test, +; Elson-Morgan's test, +550.

Conclusions

Though the presence of cancer-specific antigens in the alcohol extract of human cancer has been asserted, there has no satisfactory information as to the nature of these antigens. In the present investigation I have succeeded, by more precise methods, in isolating substances represented each by a single peak on the electrophoretic pattern. From the fact that the reactions of the sera against gastric cancer and normal gastric mucosa were not affected at all by the absorption with substances 4′ to 9′ of normal gastric mucosa and substances 7 to 9 of gastric cancer respectively, it is clear that substances α and β of gastric cancer have an antigenicity entirely

Table XA

Antiserum: Rabbit serum against alcohol extract of human gastric cancer, diluted 1 :100. Antigen: Substance α and β of human gastric cancer, doubling dilutions of stock antigen starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
0.6 mg. substances 4′ to 9′ of normal human gastric mucosa	Substance α of human gastric cancer	−	−	−	++	###	###	###	###	###	###	###
	Substance β of human gastric cancer	−	−	−	+	++	###	###	###	###	###	###
	Control antiserum											###

Table XB

Antiserum: Rabbit serum against alcohol extract of normal human gastric Mucosa, diluted 1 :100. Antigen: Substance α′ and β′ of normal human gastric mucosa, doubling dilutions of stock antigen, starting from 1 :20.

1 cc. antiserum absorbed with	Antigen	20	40	80	160	320	640	1280	2560	5120	10240	Control antigen
1.2 mg. substances 7 to 9 of human gastric cancer	Substance α′ of normal human gastric mucosa	−	−	−	−	−	±	++	###	###	###	###
	Substance β′ of normal human gastric mucosa	−	−	−	−	−	∓	++	###	###	###	###
	Control antiserum											###

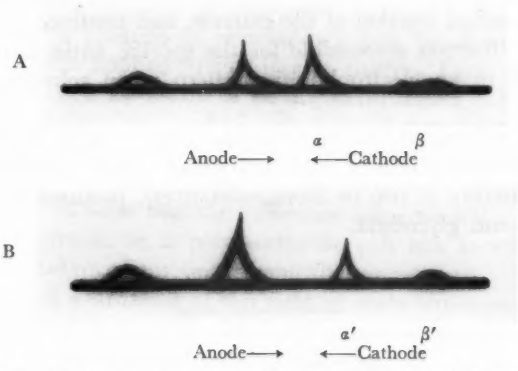

Fig. 2. Electrophoretic patterns of substances 7 to 9 of human gastric cancer (A) and substances 4′ to 9′ of normal human gastric mucosa (B), 1% solutions, carbonate buffer, pH 9.62.

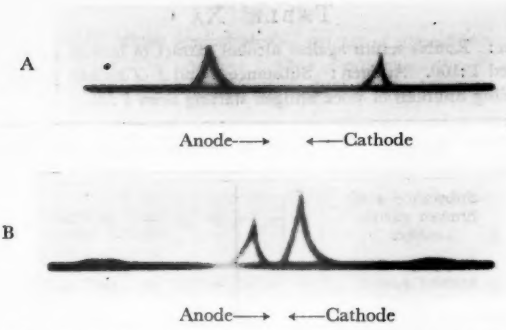

Fig. 3. Electrophoretic patterns of substance α of human gastric cancer (A) and substance α′ of normal human gastric mucosa, 1% solutions, carbonate buffer, pH 9.62.

different from that of substances α' and β' of normal gastric mucosa, and can be taken to be antigens specific to gastric cancer.

Considering the process of fractionation of the alcohol extracts and the results of qualitative analyses, these specific substances may be taken to be single substances presumably consisting of phospholipid and glycolipid.

Summary

1. The specific antigenicity demonstrated in the alcohol extract of human gastric cancer was traced by preparing rabbit antisera against alcohol extracts of human gastric cancer, normal gastric mucosa, performing complement fixation tests of these antisera, aided by absorption tests, with chemical fraction of the extracts, and continuing the fractionation until the fraction responsible for the specific antigenicity could give a single peak in an electrophoretic pattern when submitted to electrophoresis.

2. Two substances specific to human gastric cancer have been isolated. They are closely related with each other. Each substance is a single one (not a mixture of two or more substances), presumably consisting of phospholipid and glycolipid.

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Studies on the Specific Antigens of Human Gastric Cancer. III

By

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Kelling[1] and Hecht[2] have maintained that a saline extract of human cancer presents an antigenicity specific to that cancer and a protein is responsible for it. Though this was confirmed by Matsuura[3], Furusawa[4], and Yamamoto[5] in our country, Oka[6] has quite recently reported that rabbits subjected to injection of a saline extract of human gastric cancer failed to produce specific precipitin. Further, by referring to the results reported by Dochez, Heidelberger and others[7][8], Sunada and Komiyama[9] isolated from human cancer a polysaccharide-like substance showing a specific antigenicity; Murata[10] also has maintained that a water-soluble substance of human cancer, mainly consisting of polysaccharide, presents a specific antigenicity and a close antigenic relationship with the placental tissue.

With the recent progress in immunology and biochemistry modern techniques have been applied to this line of study. Thus Kidd, Mann and others[11][12] isolated by means of an ultracentrifuge a protein showing a cancer-specific antigenicity, and Macula[13] found that the nucleoprotein of a transplantable tumor is clearly different from that of the normal tissue.

Thus, opinion is much divided on the nature of specific antigens of human cancer. A view that the substance responsible for the specific antigenicity is a protein or a polysaccharide can not accepted blindly so long as the substance was not chemically pure.

In view of such a situation of this field of study attempts were made to clarify the nature of the specific antigenicity by more precise procedures, with the results to be reported in the following.

Essentially, the experiments consisted of (1) producing antisera in rabbits by injection of saline extracts of human gastric cancer, normal gastric mucosa, and placenta, (2) preparing antigens for tests from highly purified fractions of saline and aqueous extracts of the above three kinds

of tissue, and (3) performing reciprocal precipitin tests aided by reciprocal absorption tests. Especially in carrying out the serological test, I received generous guidance of Prof. Murakami (director) and members of the legomedical Institute of our university.

Experimental

Preparation of Antigens for Immunization

The specimens of gastric cancer and normal gastric mucosa were taken from the fresh excised stomachs of patients with gastric cancer and with gastric or duodenal ulcer, respectively, operated on in our clinic. They were cut into fine pieces and washed in water to remove blood. Excess water being fully removed with filter paper. 10 g. of the material was weighed out, to which was added 90 cc. of physiological saline. The mixture was shaken on a shaking apparatus for 48 hours to extract the material and centrifuged. Phenol was added to the supernatant to give a final concentration of 0.5 per cent and the solution was stored in a stoppered brown bottle in an ice chamber until used.

The specimens of placenta were taken immediately after birth in the gynecological clinic. The antigenic preparation was made and stored in the same manner as above. In order to avoid the appearance of an antibody specific to group A, tissues belonging to group O or B were used exclusively, as described in the first report.

Immunization

Healthy rabbits, weighing about 2.5 kg., of serum group O′ or β' and saliva group A (+) were used for immunization as described in the first report. Immunization was successful with 11 out of 50 rabbits. Each rabbit received 9 injections (into the ear vein) of increasing amounts of antigen, 2,3,4, . . . 9 and 10 cc., at intervals of tweice a weak. A preliminary titration was made 7 days after the last injection. If the titer was found high enough, total bleeding was made from the carotid artery. The serum separated from the blood was inactivated at 56°C. for 30 minutes, to which was added phenol to give a final concentration of 0.5 per cent, and stored in a stoppered brown bottle in an ice chamber.

Precipition Tests

Precipitin reaction was examined by ring tests, in which the amount of antiserum was held constant and antigen was varied in doubling dilution series. The results were read after 2 hours' incubation as follows: +, positive; −, negative; ±, obscure, but possibly positive. As antigenic controls, physiological saline was superposed instead of antigen; as

TABLE I

Tests Using Immune Antigens

Antigen: Doubling dillutions of the saline extract of 3 tissues.

1 cc. antiserum absorbed with	Antigen saline extract of	Saline extract	2	4	8	16	32	64	128	256	512	1624	2048	Control antigen
A. Antiserum: Rabbit serum against the saline extract of human gastric cancer														
Normal human serum 4 drops	Human gastric cancer	+	+	+	+	+	+	+	+	+	+	−	−	−
	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	+	−	−	
	Human placenta	+	+	+	+	+	+	+	+	+	−	−	−	
B. Antiserum: Rabbit serum against the saline extract of normal human gastric mucosa														
Normal human serum 4 drops	Normal human gastric mucosa	+	+	+	+	+	+	+	+	−	−	−	−	−
	Human gastric cancer	+	+	+	+	+	+	+	+	−	−	−	−	
	Human placenta	+	+	+	+	+	+	+	−	−	−	−	−	
C. Antiserum: Rabbit serum against the saline extract of human placenta														
Normal human serum 4 drops	Human placenta	+	+	+	+	+	+	+	+	+	±	−	−	−
	Human gastric cancer	+	+	+	+	+	+	+	+	+	±	−	−	
	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	±	−	−	
	Control antiserum													−

antiserum controls inactivated normal serum was used.

Absorption Tests

For tracing the specific antigenicity of human cancer, the absorption test with the 3 antisera was an important means. The necessary and sufficient amount of a fraction of each tissue to be used for absorption was determined by preliminary tests. A given amount of a fraction was added to 1 cc. of antiserum; the mixture was well rubed up with a glass rod and placed in an incubator at 37°C., being shaken every 15 minutes for a full mixing. After 2 hours the mixture was centrifuged and the supernatant was separated for use as "absorbed" antiserum.

Esperimental Results

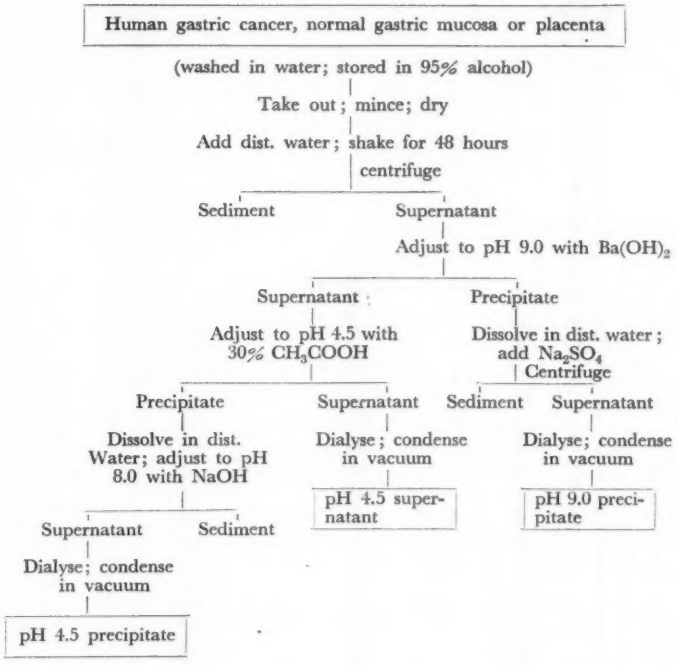
1) Tests using immune antigens

The results of precipitin tests of immune antigens with the 3 antisera are summarized in Table I (A, B, C), where it will be seen that there were no definite differences in their reactions with each antiserum among the 3 antigens. This may be explained as follows: A tissue has species specificity as well as organ specificity, and the former is generally more influential, if blood or body fluids in mixed with the tissue extract, the species specificity becomes further influential. Thus, normal serum (having the predominant species specificity over all other human tissues) was added to each antiserum with a capillary pipette at a rate of 4 drops per 1 cc. of the antiserum in order to absorb species-specific antibodies completely (i.e., in order to specialize the antiserum). The resultes of tests in which each antiserum thus specialized was used instead of the untreated antiserum are shown in the left corner of the main part of Table I (A, B, C), (surrounded by heavy lines). It will be seen that each antigen reacted most strongly with its homologous antiserum. Therefore, the next step was to trace the specificity and similarity of each antigen by chemically separating it into various fractions and making precipitin tests with each of these fractions.

2) Tests using crude fractions as antigen

The 3 tissues that had been stored in 95 per cent alcohol were extracted in distilled water, and (1) the precipitates produced from each extract by barium hydroxide at pH 9.0, (2) the precipitates produced by 30 per cent acetic acid at pH 4.5, and (3) the supernatant after the above treatment with acetic acid, were purified as shown in Table II and used as antigens for precipitin tests. The 3 antisera to be used had previously been absorbed on saturation with normal human serum. The results of tests with each fraction of pH 9.0 precipitates are shown in Table III (A, B, C). It

TABLE II



will be seen (1) that gastric cancer and normal gastric mucosa did not react with the rabbit serum against the saline extract of human placenta (hereafter to be referred to as anti-placenta serum), (2) that placenta did not react with the rabbit serum against the saline extract of normal human gastric mucosa (hereafter to be referred to as anti-normal gastric mucosa serum) and the rabbit serum against the saline extract of human gastric cancer (hereafter to be referred to as anti-gastric cancer serum), and (3) that accordingly the fraction of pH 9.0 precipitates was highly organ-specfic. After absorption with 15 mg. of the pH 9.0 precipitate fraction of normal gastric mucosa, the anti-gastric cancer serum reacted only with gastric cancer as shown in the upper left corner (surrounded by heavy lines) of Table III A, whereas the anti-normal gastric mucosa serum absorbed with 20 mg. of the pH 9.0 precipitate fraction of gastric cancer did not react with any of the 3 antigens, which results are suggestive of the presence of antigens specific to human gastric cancer.

Precipitin tests with the pH 4.5 precipitate fraction of each tissue as antigen yielded the results shown in Table IV (A, B, C), where it will be

TABLE III

Tests Using Crude Fractions as Antigen

Antigen : The pH 9.0 percipitates of 3 Tissues ×50–×7000.

1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1000	2000	3000	4000	5000	6000	7000	Control antigen
A. Antiserum : Rabbit serum against the saline extract of human gastric cancer (previously absorbed with normal human serum)														
15 mg. pH 9.0 precipitate of normal human gastric mucosa	Human gastric cancer	+	+	+	+	+	+	−	−	−	−	−	−	−
	Normal human gastric mucosa	+	+	+	−	−	−	−	−	−	−	−	−	
	Human placent	−	−	−	−	−	−	−	−	−	−	−	−	
B. Antiserum : Rabbit serum against the saline extract of normal human gastric mucosa (ditto)														
20 mg. pH 9.0 precipitate of human gastric cancer	Normal human gastric mucosa	+	+	+	+	+	+	+	−	−	−	−	−	−
	Human gastric cancer	+	+	+	+	−	−	−	−	−	−	−	−	
	Human placenta	−	−	−	−	−	−	−	−	−	−	−	−	
C. Antiserum : Rabbit serum against the saline extract of human placenta (ditto)														
	Human placenta	−	−	−	−	−	−	−	−	−	−	−	−	−
	Human gastric cancer	−	−	−	−	−	−	−	−	−	−	−	−	
	Normal human gastric mucosa	−	−	−	−	−	−	−	−	−	−	−	−	
	Control antiserum													−

TABLE IV

Tests Using Crude Fractions as Antigen

Antigen : The pH 4.5 precipitates of 3 Tissues ×50–×8000.

1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1000	2000	4000	5000	6000	7000	8000	Control antigen
A. Antiserum : Rabbit serum against the saline extract of human gastric cancer (previously absorbed with normal human serum)														
20 mg. pH 4.5 precipitate of normal human gastric mucosa	Human gastric cancer	+	+	+	+	+	+	−	−	−	−	−	−	−
	Normal human gastric mucosa	+	+	+	+	+	−	−	−	−	−	−	−	
	Human placenta	−	−	−	−	−	−	−	−	−	−	−	−	
B. Antiserum : Rabbit serum against the saline extract of normal human gastric mucosa (ditto)														
20 mg. pH 4.5 precipitate of human gastric cancer	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	−	−	−	−
	Human gastric cancer	+	+	+	+	+	+	−	−	−	−	−	−	
	Human placenta	−	−	−	−	−	−	−	−	−	−	−	−	
C. Antiserum : Rabbit serum against the saline extract of human placenta (ditto)														
	Human placenta	+	−	−	−	−	−	−	−	−	−	−	−	−
	Human gastric cancer	−	−	−	−	−	−	−	−	−	−	−	−	
	Normal human gastric mucosa	−	−	−	−	−	−	−	−	−	−	−	−	
	Control antiserum													−

TABLE V

Tests Using Curde Fractions as Antigen

Antigen: The pH 4.5 supernatantes of 3 Tissues ×50–×10000.

1 cc. antiserum absorbed with	Antigen	50	100	200	300	500	1000	2000	4000	6000	7000	9000	10000	Control antigen
A. Antiserum: Rabbit serum against the saline extract of human gastric cancer (previously absorbed with normal human serum)														
First: 20 mg. pH 4.5 supernatant of human placenta	Human gastric cancer	+	+	+	+	+	+	+	+	+	+	+	−	−
Second: 20 mg. pH 4.5 supernatant of normal human gastric mucosa	Normal human gastric mucosa	+	+	+	+	+	+	+	+	−	−	−	−	
	Human placenta	+	+	+	+	+	−	−	−	−	−	−	−	
B. Antiserum: Rabbit serum against the saline extract of normal human gastric mucosa (ditto)														
First: 4 mg. pH 4.5 supernatant of human placenta	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	+	+	−	−
Second: 19 mg. pH 4.5 supernatant of human gastric cancer	Human gastric cancer	+	+	+	+	+	+	+	−	−	−	−	−	
	Human placenta	+	−	−	−	−	−	−	−	−	−	−	−	
C. Antiserum: Rabbit serum against the saline extract of human placenta (ditto)														
First: 4 mg. pH 4.5 supernatant of normal human gastric mucosa	Human placenta	+	+	+	−	−	−	−	−	−	−	−	−	−
Second: 18 mg. pH 4.5 supernatant of human gastric cancer	Human gastric cancer	+	+	±	−	−	−	−	−	−	−	−	−	
	Normal human gastric mucosa	±	−	−	−	−	−	−	−	−	−	−	−	
	Control antiserum													−

found that the relations among the 3 antigens were quite similar to those in the case of the pH 9.0 precipitate fraction.

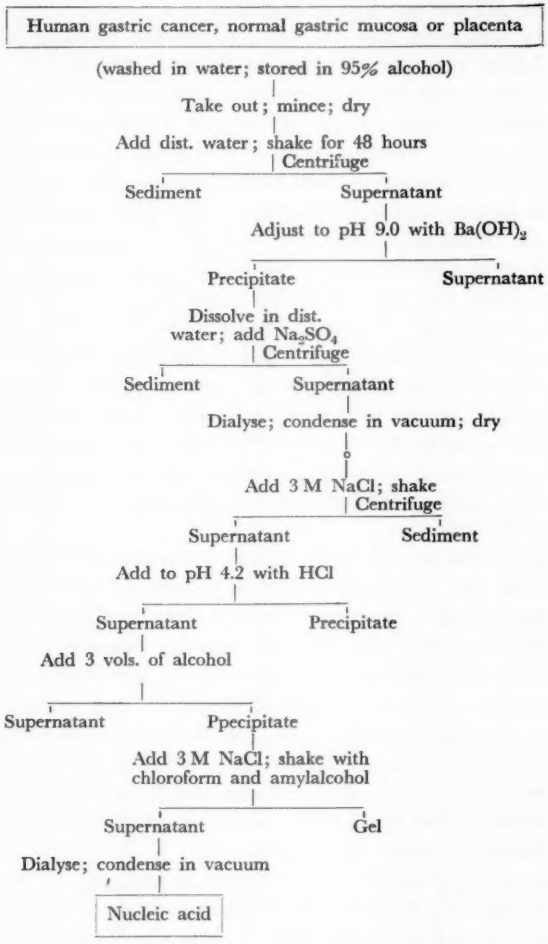
Precipitin tests with the pH 4.5 supernatant fraction of each tissue as antigen yielded the results shown in Table V (A, B, C). Comparing the results of tests using the anti-gastric cancer serum with those using the antiserum absorbed with 20 mg. of the pH 4.5 supernatant fraction of normal human gastric mucosa (shown in the left corners of Table V A, surrounded by broken lines), it will be understood that gastric cancer had some antigens in common with placenta. The tests using the anti-normal gastric mucosa serum absorbed with the pH 4.5 supernatant fraction of placenta yielded the results shown in the aera surrounded by heavy lines (Table V B). while the tests using the same antiserum but absorbed with the pH 4.5 supernatant of gastric cancer gave the results shown in the area surrounded by broken lines. The differences between both results indicate that gastric cancer and normal human gastric mucosa had some different antigens. As for tests with the anti-placenta serum (Table VC), the differences between the results before and after being absorbed with the pH 4.5 supernatant of normal gastric mucosa indicate that gastric cancer had some antigens in common with placenta. (Table VC, surrounded area by heavy lines)

From the results stated above it may be assumed that human gastric cancer has some specific antigens and is related to placenta more closely than to normal gastric mucosa. Judging from the chemical procedures through which the 3 fractions of each tissue (9 fractions in all) were obtained, however, each fraction may possibly be a mixture of several substances. Therefore, from the results of precipitin tests using such fractions as antigen, no decisive conclusions can not be drawn concerning the nature of antigens specific to human gastric cancer and of antigens common to this cancer and placenta. Thus, the next step was to isolate certain chemically purer substances from the 3 tissues stored in alcohol and make precipitin tests using these substances as antigen.

3) Tests using a nucleic acid fraction as antigen

A nucleic acid fraction was prepared from each of the 3 tissues stored in 95 per cent alcohol by the method as shown in Table VI. The results of qualitative tests of this preparation are as follows: phosphorus test, ++; Molish's test, ±; biuret test, ±; Bial's test, +. The results of precipitin tests using as antigen the nucleic acid fraction of each tissue are summarized in Table VII (A, B, C), where it will be seen that definite difference in their reaction with the anti-gastric cancer serum was presented between gastric cancer and normal gastric mucosa (Table VII A), while gastric cancer and placenta still reacted with the antiserum absorbed with normal gastric mucosa (shown in the left corners of A, surrounded by heavy lines),

TABLE VI



thus indicating that the gastric cancer nucleic acid has some antigens in common with placenta as well as highly specific antigens.

The anti-normal gastric mucosa serum reacted positively with all the 3 tissues, though differently in intensity (Table VII B), but did not react with any of the 3 antigens after being absorbed with the gastric cancer nucleic acid.

The anti-placenta serum reacted with gastric cancer more strongly

TABLE VII

Tests Using a Nucleic Acid Fraction as Antigen

Antigen: The nucleic acids of 3 Tissues ×50–×7000.

1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1000	2000	3000	4000	5000	6000	7000	Control antigen
A. Antiserum: Rabbit serum against the saline extract of human gastric cancer (previously absorbed with normal human serum)														
10 mg. nucleic acid of normal human gastric mucosa	Human gastric cancer	+	+	+	+	+	–	–	–	–	–	–	–	–
	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	+	+	–	
	Human placenta	+	+	+	+	–	–	–	–	–	–	–	–	
B. Antiserum: Rabbit serum against the saline extract of normal human gastric mucosa (ditto)														
10 mg. nucleic acid of human gastric cancer	Normal human gastric mucosa	+	+	+	+	+	+	+	+	–	–	–	–	–
	Human gastric cancer	+	+	+	+	+	–	–	–	–	–	–	–	
	Human placenta	+	+	+	–	–	–	–	–	–	–	–	–	
C. Antiserum: Rabbit serum against the saline extract of human placenta (ditto)														
First: 10 mg. nucleic acid of normal human gastric mucosa Second: 10 mg. nucleic acid of human gastric cancer	Human placenta	+	+	+	+	+	+	+	+	–	–	–	–	–
	Human gastric cancer	+	+	+	+	+	+	+	–	–	–	–	–	
	Normal human gastric mucosa	+	+	+	+	–	–	–	–	–	–	–	–	
	Control antiserum													–

than with normal gastric mucosa, and after absorption with normal gastric mucosa nucleic acid it reacted with both placenta and gastric cancer, which results demonstrate some common antigenicity between the nucleic acids of placenta and gastric cancer (Table VII C, surrounded area by heavy lines).

In summary, it may be concluded that the gastric cancer nucleic acid has some specific antigenicity, since it is incapable of being absorbed on full saturation with the normal gastric mucosa nucleic acid, and is related with placenta more closely than with normal gastric mucosa.

4) Tests using a carbohydrate fraction as antigen

A carbohydrate fraction was prepared from each tissue stored in 95 per cent alcohol by the method shown in Table VIII, and gave the following qualitative reactions: Molisch's test, +; biuret, phosphorus, and Bial's test, −. The results of precipitin tests using as antigen this carbohydrate fraction of each tissue are summarized in Table IX (A, B, C), where it will be seen that the anti-gastric cancer serum reacted with normal gastric mucosa and gastric cancer strongly and almost equally, but, after absorption with the normal gastric mucosa carbohydrate, only slightly with gastric cancer and placenta (Table IX A surrounded area by heavy lines).

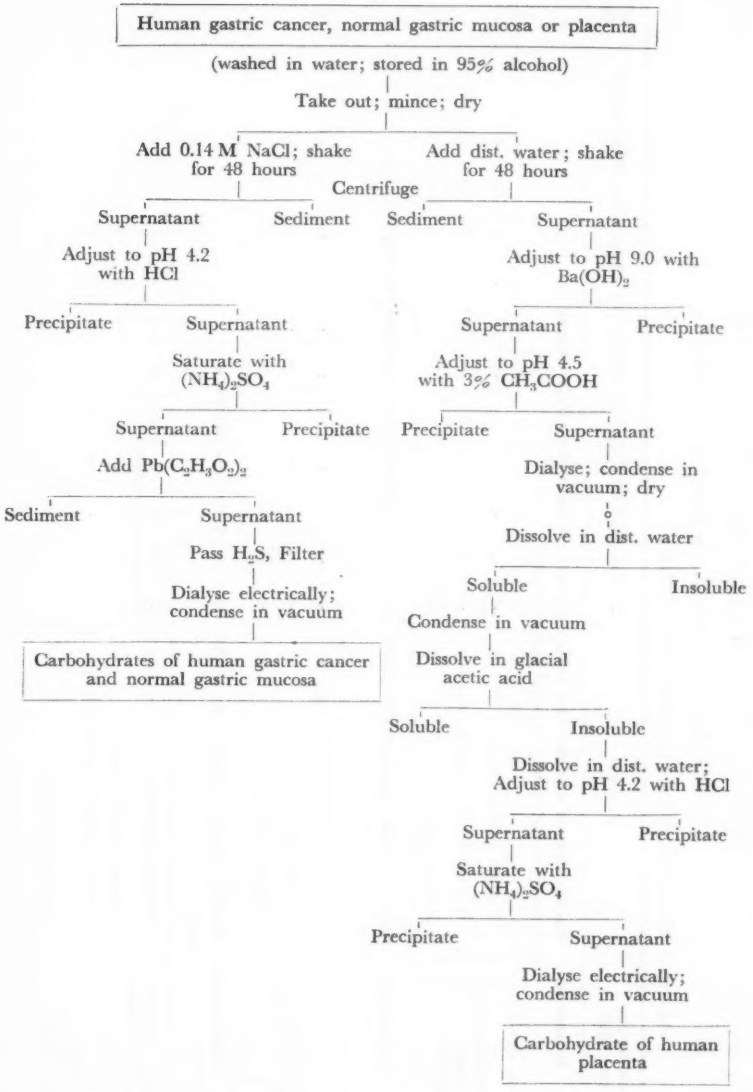
Now, when absorbed with the gastric cancer carbohydrate, the anti-normal gastric mucosa serum reacted only with normal gastric mucosa (Table IX B). The reactions of the anti-placenta serum with gastric cancer and normal gastric mucosa were the same in intensity (Table IX C). After absorption with the normal gastric mucosa, the antiplacenta serum reacted only with placenta slightly (surrounded area by heavy lines), after further absorption with the gastric cancer carbohydrate, this antiserum reacted only with placenta as before and a little more slightly (surrounded area by broken lines).

Considering these results, the antigenic specificity of the gastric cancer carbohydrate seems to be low, antigenic differences of carbohydrate between gastric cancer and normal gastric mucosa being indistinct, though there is something in common between the carbohydrates of gastric cancer and placenta.

5) Tests using a glucidamin fraction as antigen

A glucidamin (a protein) fraction was prepared from each of the 2 tissues, gastric cancer and normal gastric mucosa, the purification from placenta being unable on account of shortage of material (Table X). Each fraction gave the following qualitative reactions: biuret and Molisch's tests, +; Bial's and phosphorus tests, −. The results of precipitin tests using as antigen this glucidamin fraction of each tissue are shown in Table XI (A, B, C). In A it will be seen that the anti-gastric cancer serum reacted with both antigens almost equally, but only with gastric cancer after ab-

TABLE VIII



sorption with the normal gastric mucosa glucidamin, results being suggestive of the specific antigenicity of gastric cancer glucidamin (surrounded area

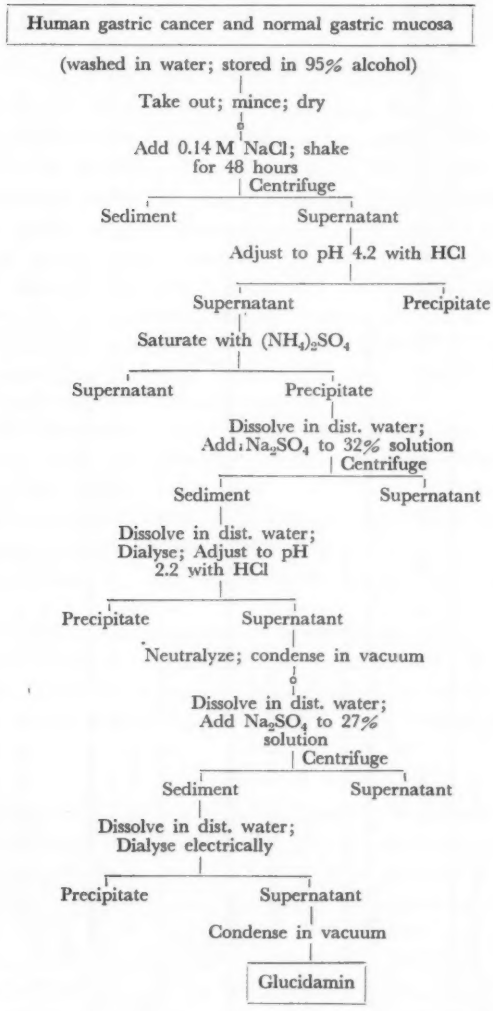
TABLE IX

Tests Using a Carbohydrate Fraction as Antigen

Antigen: The carbohydrates of 3 Tissues ×50–×200,000.

1 cc. antiserum absorbed with	Antigen	50	400	800	1000	3000	4000	10000	20000	40000	80000	100000	200000	Control antigen
A. Antiserum: Rabbit serum against the saline extract of human gastric cancer (previously absorbed with normal human serum)														
5 mg. carbohydrate of normal human gastric mucosa	Human gastric cancer	+	+	+	+	+	+	+	+	+	+	+	−	−
	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	+	±	−	
	Human placenta	+	+	+	−	−	−	−	−	−	−	−	−	
B. Antiserum: Rabbit serum against the saline extract of normal human gastric mucosa (ditto)														
5 mg. carbohydrate of human gastric cancer	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	−	−	−	−
	Human gastric cancer	+	+	+	+	+	+	+	−	−	−	−	−	
	Human placenta	+	+	−	−	−	−	−	−	−	−	−	−	
C. Antiserum: Rabbit serum against the saline extract of human placenta (ditto)														
First: 5 mg. carbohydrate of normal human gastric mucosa Second: 5 mg. carbohydrate of human gastric cancer	Human placenta	+	+	+	+	+	+	+	+	−	−	−	−	−
	Human gastric cancer	+	+	+	+	+	−	−	−	−	−	−	−	
	Normal human gastric mucosa	+	+	+	+	+	−	−	−	−	−	−	−	
	Control antiserum													−

TABLE X



by heavy lines). On the other hand, the anti-normal gastric mucosa serum reacted with both antigens, but with neither of them after absorption with gastric cancer glucidamin (Table XIB). The anti-placenta serum reacted with gastric cancer more strongly than with normal gastric mucosa.

In summary, it may be concluded from these results that gastric cancer

TABLE XI

Tests Using a Glucidamin Fraction as Antigen

Antigen: The Glucidamins of 2 Tissues ×50–×64,000.

1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1000	2000	4000	8000	16000	32000	64000	Control antigen
A. Antiserum: Rabbit serum against the saline extract of human gastric cancer (previously absorbed with normal human serum)														
10 mg. Glucidamin of normal human gastric mucosa	Human gastric cancer	+	+	+	+	+	+	+	+	+	±	−	−	−
	Normal human gastric mucosa	+	+	+	+	+	+	+	+	+	+	−	−	
B. Antiserum: Rabbit serum against the saline extract of normal human gastric mucosa (ditto)														
10 mg. Glucidamin of human gastric cancer	Normal human gastric mucosa	+	+	+	+	+	+	+	−	−	−	−	−	−
	Human gastric cancer	+	+	+	+	−	−	−	−	−	−	−	−	
C. Antiserum: Rabbit serum against the saline extract of human placenta (ditto)														
	Human gastric cancer	+	+	+	+	−	−	−	−	−	−	−	−	−
	Normal human gastric mucosa	+	+	±	−	−	−	−	−	−	−	−	−	
	Control antiserum													−

glucidamin has some specific antigens, part of which are in common with placenta glucidamin.

Discussion and Conclusions

A general survey of the results obtained from the precipitin tests using nucleic acid, carbohydrate, and glucidamin as antigen will reveal us that the reaction of each Fraction of human gastric cancer with an antiserum was always different from the reaction of the corresponding fraction of normal human gastric mucosa, and especially in the case of the nucleic acid fraction human gastric cancer showed the most remarkable specific antigenicity and a remarkably close relationship with human placenta (Table VII). Considering the process of fractionation shown in Table VI, the nucleic acid preparation seems to have mainly consisted of ribonucleic acid, which is an interesting thing in view of the fact that ribonucleic acid is closely related with cellular protein metabolism.

The specific antigenicity of human gastric cancer and a close relation ship of the cancer with placenta may be taken to have appeared also in the glucidamin fraction (Table XI).

The carbohydrate fraction differed from the other two, in that no marked specific antigenicity of human gastric cancer was presented, there being found only something in common between gastric cancer and placenta (Table IX).

Thus, the specific antigenicity of human gastric cancer does not seem to be restricted to a special fraction, but is presented by several fractions to varying degrees. Thus, serologically, the specificity is highest in the nucleic acid, moderate in the glucidamin, and lowest in the carbohydrate fraction.

Summary

With a view to tracing the specific antigenicity of human gastric cancer a series of experiments were carried out, which essentially consisted of (1) producing antisera in rabbits by injection of saline extracts of human gastric cancer, normal gastric mucosa, and placenta, (2) preparing several purified fractions of the above three kinds of tissue, which were used as antigens for precipitin tests, and (3) performing reciprocal precipitin tests aided by reciprocal absorption tests. It has been demonstrated that the nucleic acid fraction of human gastric cancer presented the highest antigenic specificity, the glucidamin fraction a modertae, and the carbohydrate fraction least specificity.

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Electromyographic Study on Various Brain Diseases Undergoing Operations

By

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(Received for publication, February 19, 1955)

Studies of E.M.G. have made a great advance recently[1)2)]. It has become evident that the E.M.G. is significant for the diagnosis of diseases on the lower motor neuron. Clinical application of E.M.G. on disease of the upper motor neuron is, however, not clarified. Sargent[3)] expressed that E.M.G. has only diagnostic significance on disease of the lower motor neuron. It is conceivable that some abnormal findings will be detected on disease of the upper motor neuron by E.M.G.

The author made an electromyographical study on cerebral disease exemplify its diagnostic significance.

Experimental

Materials and Method

5 cases of brain tumors, 2 cases of cerebral trauma, 4 cases of cerebellopontine tumor, 3 cases of hydrocephalus and 4 cases of cerebral abscess were examined. E.M.G. from the extremities were recorded by a concentric needle electrode with an oscillograph equipped with a R–C 4 stage amplifier and plate electrode with an ink-writing electroencephalograph, at rest, during passive extension, voluntary contraction and on foot. The pattern of wave and fluctuation of the impulse interval of single motor unit was observed. According to the pathology of the patient, various changes were observed on the E.M.G.

Results and Discussion

Spontaneous discharge at rest and during passive extension

5 cases showed spontaneous discharge at rest. The waves had an amplitude over 200 μV, and impulse interval was relatively stabilized as in normal wave (Fig. 1). These findings are obviously different from fibrillation voltage or fasciculation voltage which appears in case of disease

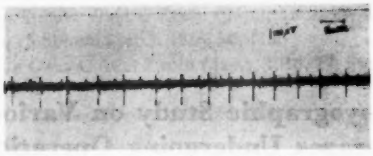

Fig. 1. Cerebral abscess. M. biceps brachii. at rest. Spontaneous discharge.

with motor neuron. 7 cases showed discharge during passive extension. The E.M.G. showed normal waves, which can be regarded waves due to severe spastic paralysis. The difference of the wave due to localization of the tumor could not be recognized.

Synchronization voltage

Abnormal E.M.G. which showed synchronization voltage was seen in 2 cases of cerebral tumor and one case of trauma to the brain during active contraction. The wave showed spike discharge with large amplitude 2–3 mV. Even at maximum contraction, it didn't show interference voltage, but was recognized as single oscillation (Fig. 2). The amplitude showed waning and waxing (Fig. 3), and simultaneous charge (Fig. 4). From the above findings, the E.M.G. can be regarded as synchronization

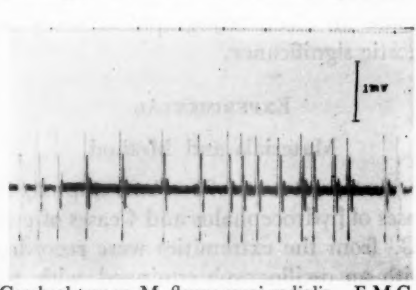

Fig. 2. Cerebral tumor. M. flexor carpi radialis. E.M.G. during maximum contraction. No interfering wave is formed.

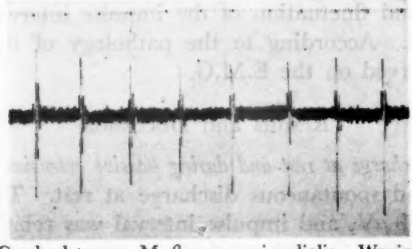

Fig. 3. Cerebral tumor. M. flexor carpi radialis. Waxing and waning are recognized.

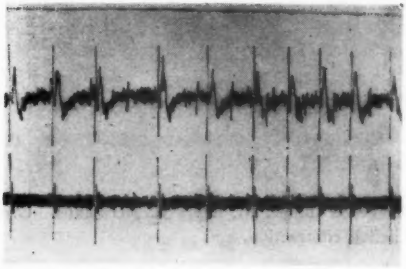

Fig. 4. Cerebral tumor. 2 leads in m. flexor carpi radialis. Synchronization is seen during active contraction.

voltage[4)5)]. Synchronization voltage is said to appear only in case of destruction and degeneration of cells in the anterior horn in the spinal cord.

It is interesting that it was demonstrated in patients with cerebral diseases. 2 of 3 cases had tumors in the temporal lobe, and one was suffering from depression fracture of the skull of the temporal region. Both showed compression to the motor area. Clinical symptoms of motor paresis were shown in both cases, whereas hemiplegia due to bleeding into the internal capsula didn't show synchronization voltage. Therefore, it seems that there is no direct relationship between the pathology of subcortical pyramidal tract and synchronization. Synchronization voltage which occurrs in case of cerebral disease may appear only when the cortical motor area is compressed. This phenomenon is not only interesting to consider the mechanism of synchronization voltage, but also has diagnostic significance.

Grouping voltage

7 cases among those with cerebellar tumor, cerebellopontine tumor and hydrocephalys showed grouping voltage during voluntary contraction. All showed cerebellar ataxia. Grouping voltage was determined not only from the wave, but also from measuring the approach of spike discharge from different motor unit by Kawamura's method[6)]. The degree of grouping and strength of motor ataxia was found in parallel, while in those with motor paresis, grouping voltage was never found. It seems that grouping voltage is an important electromyographic finding in cerebellar ataxia.

E.M.G. during standing

According to the observations of E.M.G. of the lower extremities during standing, those patients with cerebellar ataxia showed participation of muscles which did not work in healthy persons. Action potentials of the muscle were not stabilised. E.M.G. was changeable in case of Romberg's syndrome (Fig. 6).

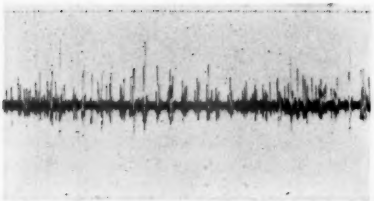

Fig. 5. Cerebellar tumor. M. flexor carpi radialis. Grouping voltage is seen during active contraction.

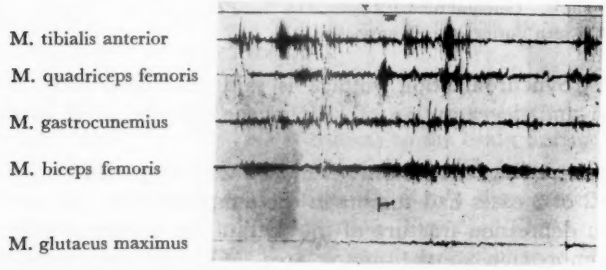

Fig. 6. Electromyogram on foot, taken by 8 elements ink writing electromyograph.

Fluctuation of the impulse interval of single motor unit

In those patients with motor paresis, the impulse interval are usually stabilized, while in those with motor ataxia, it fluctuates extensively. Grouping voltage and the interval discharge have a close relationship as being pointed out by Kawamura during the electromyographic observation in Graves' disease[6].

Conclusion

Various abnormal findings in E.M.G. were recognized in those patients with disease of the upper motor neuron. The author believes that the diagnostic significance of E.M.G. will increase with future studies.

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Activity Ratio of Adrenaline to Noradrenaline in Various Colour Reactions

By

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(Received for publication, March 1, 1955)

In estimating adrenaline content of the adrenal medullary tissue or of the adrenal venous blood, various colour reactions have been applied, adrenaline being used as the reference standard. However, it is generally accepted that noradrenaline is contained besides adrenaline in the adrenal medulla or in the adrenal venous blood. In this connection it is of great interest to clarify the colorimetric activity of noradrenaline in various colour reactions.

Experimental

Methods and Results

The permanganate, the corrosive sublimate, the iodine, the phosphotungstic acid and the arsenomolybdic acid methods found their application.

Synthetic L-adrenaline and DL-noradrenaline (Sankyo) were used as the samples. These were dissolved with N/10 HCl in 1:1,000, and further diluted if necessary. Using the respective methods, a known amount of adrenaline or noradrenaline was coloured and the colour density of noradrenaline was compared with that of adrenaline. No difference was causable by employing L-noradrenaline (Sterling Winthrop) instead of DL-noradrenaline.

Experimental results are tabulated.

The permanganate method:

The procedure used in this test was almost the same as that described by Zanfrognini[1]. In the present studies, the colour reaction was performed at constant temperature (20°C) and pH (6.0) to avoid effects causable by changes of temperature and pH. The colour tint of noradrenaline after oxidation with the permanganate reagent was approximately similar to that of adrenaline.

The colorimetric activity ratios of adrenaline to noradrenaline ob-

TABLE I

Colorimetric Activity Ratios of Adrenaline to Noradrenaline in Various Chemical Methods

No. of exp.	Permanganate method	Corrosive sublimate method	Iodine method	Phosphotungstic acid method	Arsenomolybdic acid method
1	1.16:1	1.30:1	2.3:1	2.20:1	7.2:1
2	1.16:1	1.32:1	2.3:1	2.20:1	7.8:1
3	1.15:1	1.29:1	2.3:1	2.18:1	7.8:1
4	1.17:1	1.32:1	2.5:1	2.11:1	7.9:1
5	1.17:1	1.30:1	2.3:1	2.15:1	7.1:1
6	1.17:1	1.31:1	2.5:1	2.18:1	7.6:1
7	1.16:1	1.32:1	2.3:1	2.15:1	8.0:1
8	1.16:1	1.28:1	2.3:1	2.20:1	7.6:1
9	1.15:1	1.32:1	2.3:1	2.19:1	7.6:1
10	1.14:1	1.29:1	2.5:1	2.17:1	7.3:1
Mean	**1.16:1**	**1.31:1**	**2.4:1**	**2.17:1**	**7.6:1**

tained in 10 experiments were 1.14:1–1.17:1, the average being 1.16:1.

The corrosive sublimate method:

The method of Suto and Inouye[2] (modified by Kojima[3]) was applied. In this reaction, the colour tint of noradrenaline after oxidation was mostly similar to that of adrenaline.

The activity ratios of adrenaline to noradrenaline obtained in 10 experiments were 1.28:1–1.32:1, the average being 1.31:1.

The iodine method:

This colour reaction was carried out according to the description of Euler.[4] In this iodine method, however, it was noticed that the colour tint differed remarkably between adrenaline and noradrenaline; that is, brownish red in adrenaline and purplish red in noradrenaline. Furthermore, the colour development of noradrenaline varied correspondingly to the changes of the reaction temperature.

Therefore the following procedure was contrived: 1) Colorimetric comparisons were made after adding to noradrenaline some amount of adrenaline enough to make the colour tint similar to that of adrenaline. 2) The colour reaction was carried out at the constant temperature of 20°C in order to remove the effects causable by the changes of the reaction temperature.

The activity ratios of adrenaline to noradrenaline obtained in 10 experiments were 2.3:1–2.5:1, and the mean value was 2.4:1. The somewhat greater variability of these values seems to be due to the difference of colour tint between adrenaline and noradrenaline and to the procedure

contrived to reduce it.

The phosphotungstic acid method:

The method of Folin, Cannon and Denis[5] was used. In my experiments, however, the colour densities of adrenaline and noradrenaline were compared colorimetrically directly without using uric acid as the standard.

The colour of noradrenaline obtained in this reaction had a little more dark-brownish tone than that of adrenaline. Nevertheless, the colorimetric comparison was practically not so difficult.

The activity ratios of adrenaline to noradrenaline were found in 10 experiments to be 2.11:1–2.20:1, and the average was 2.17:1.

The arsenomolybdic acid method:

This colour reaction was performed, using the method of Bloor and Bullen[6]. The colour tint of adrenaline and noradrenaline obtained in this reaction was approximately similar. The colorimetric activity ratios of adrenaline to noradrenaline were estimated to be 7.1:1–8.0:1, the average being 7.6:1.

The considerable variability of these values may be due to the complexity of procedure itself.

Discussion

In the present investigation, the colorimetric activity ratio of adrenaline to noradrenaline was estimated by the following 5 chemical methods—the permanganate, the corrosive sublimate, the iodine, the phosphotungstic acid and the arsenomolybdic acid methods.

From my experimental results it is assumed that the disharmony of adrenaline estimates yielded by various methods as was found by the previous investigators (Kojima *et al.*[7], Hatano[8] and Arai[9]) may be due to the difference of the activity ratio of adrenaline to noradrenaline in each method. This was already suggested by Suzuki, Nakamura and Ninagawa[10] in their study on the biological activity ratio of adrenaline to noradrenaline. We should obtain lower adrenaline estimates in the chemical methods in which the activity ratio of adrenaline to noradrenaline is higher, if only noradrenaline is contained besides adrenaline in the adrenal medulla. However, experimental results reported by Kojima *et al.*, Hatano and Arai are not fully explainable from my present data. It must be taken into account that the activity ratio of adrenaline to noradrenaline in various chemical methods may be influenced by the variations of the reaction temperature and of pH. Therefore this problem is to be reinvestigated, taking the experimental conditions into consideration.

Some authers reported the activity ratio of adrenaline to noradrenaline in Shaw's arsenomolybdic acid method. In the experiments of Shaw,[11] it was found to be 16:1. By the hand

of West[12], it was estimated to be 13.5 :1. And it was estimated by Verly[13] to be about 5 :1. Gaddum, Peart and Vogt[14], on the other hand, obtained just the same value as Shaw.

Summary

Activity ratios of adrenaline to noradrenaline in various chemical methods, such as the permanganate, the corrosive sublimate, the iodine, the phosphotungstic acid and arsenomolybdic acid methods were estimated.

The mean value (adrenaline : noradrenaline) obtained was 1.16:1 in the permanganate method, 1.31:1 in the corrosive sublimate method, 2.4:1 in the iodine method, 2.17:1 in the phosphotungstic acid method and 7.6:1 in the arsenomolybdic acid method.

I hereby express my sincere gratitude to Prof. T. Suzuki for his interest in this work.

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Histopathologic Studies on Chronic Cystic Mastitis, with Special Reference to Malignant Degeneration

By

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(Received for publication, March 5, 1955)

It is generally accepted that malignant degeneration may occur in chronic cystic mastitis. There is, however, a large discrepancy in its frequency among authors. Recently, not a few workers such as Kuru[1], Kaieda[2], and Foote and Stewart[3] have pointed out a large rate of development of mammary cancer from this disease, and the significance of this disease as a precancerous lesion has been emphasized by many authors.

Following Sato[4], who studied 23 cases of chronic cystic mastitis in our clinic, I made histopathologic investigations on the cases encountered thereafter together with cases of mammary cancer, with special reference to malignant degeneration of chronic cystic mastitis, with the results to be reported here.

Experimental

Material and Method

The material used was a total of 134 breasts out of 154 breasts operated for mammary tumors in our clinic since April, 1946, consisting of (1) 100 breasts in 69 cases of chronic cystic mastitis, and (2) 34 breasts in 34 cases diagnosed clinically and upon microscopic examination as cases of mammary cancer.

Examination of specimens. Excised specimens were examined macroscopically, and several to 26 blocks were taken from each specimen, from which serial sections were prepared when necessary. Preparations were stained with hematoxylin and eosin and by van Gieson's method in all cases, and with Mallory's connective tissue stain or by Unna's orcein method (for elastic tissue) in some cases.

Macroscopic Findings and Histologic Features of Chronic Cystic Mastitis

The characteristic features of 100 breasts in chronic cystic mastitis

were diffuse fibrosis and cyst formation. Macroscopically, these glands were mostly disk-like or somewhat semispherical in shape; there was no sharp boundary between the breast proper and a tumor. On the cut surface fibrosis was manifest even to the naked eye, and milk-white, pink-white or gray-white in color, bearing yellow fatty tissue scatterlingly. In areas of fibrosis one to several nodules were not infrequently felt: 43 solid nodules in 35 breasts (35.0 per cent), and 57 cystic ones in 27 breasts (27.0 per cent). Sato[4] points out that the frequency of occurrence of cases with remarkable cyst formation is low in our country. My findings agree with this.

These 43 solid nodules were of the order of magnitude of from a grain of rice to the tip of a thumb, could be perceived as such by touching, and included 24 nodules of fibroadenoma in 21 breasts, 13 nodules of high degree of epithelial proliferation or of early malignant degeneration in 11 breasts, and 6 nodules of partly remarkable fibrosis in 5 breasts (Table I). Though exact distinction of fibroadenoma from a high degree of epithelial proliferation and early malignant degeneration necessitated histologic investigations,

TABLE I

Histologic Features of Nodules Perceived by Touch in Tumors

Size \ Histologic features	Solid			Cystic
	Epith. prolifn. or early malignant degeneration	Fibroadenoma	Localized prolifn. of connect. tissue	
Lager than small finger tip	3	5	0	6
Of the order of small finger tip to red bean	2	3	1	8
Smaller than red bean	8	16	5	43
Total	13	24	6	57

8 fibroadenoma larger than a red bean could be diagnosed as such even macroscopically: They were spherical or oval in form, encapsuled, and definitely outlined (lobulation being perceived in 5 nodules); on the other hand, 5 nodules of high degree of epithelial proliferation or early malignant degeneration could be clearly distinguished from those of fibroadenoma when they were larger than a red bean in that they were very poorly outlined, with only slight fibrosis, and softer than those of fibroadenoma.

The magnitude of macroscopic cysts was diversed as shown in Table I. In 1 breast there was found immediately beneath the nipple a cyst of a pigeon's egg in size, part of its wall projecting into the cavity to form a

tumor of the size of the tip of a thumb (Fig. 1), while the remaining 56 cysts were of the size of the tip of a forefinger or thumb at the largest. Multiple cysts were found in only 6 breasts, the rate of occurrence (6.0 per cent) being much lower than that reported in western countries.

Fig. 1. A large cyst immediately beneath the nipple and intracystic papilloma.

Histologically, 14 cysts larger than a red bean consisted of 3 cysts with intraductal epithelial proliferation, 2 pale epithelium lining cysts, 4 cysts with a layer of compressed epithelium, and 5 cysts devoid of epithelium, thus showing a tendency of less frequent formation of macroscopic cysts with epithelial proliferation.

Originally "chronic cystic mastitis" is a general term for diseases the characteristic lesions of which are diffused fibrosis and epithelial cyst formation, macroscopic cyst formation was found in only 27.0 per cent of the 100 breasts examined, though diffuse fibrosis was recognized in all the breasts. In 35.0 per cent of the cases localized nodules were noticed in diffuse fibrosis, mostly of fibroadenoma, frequently of epithelial proliferation or early malignant degeneration, and sometimes of partly remarkable fibrosis. It was found that this partly intense fibrosis was perceived by touch as localized nodules.

Malignant Features of Chronic Cystic Mastitis

Malignant degeneration of chronic cystic mastitis was found in 10 out of the 100 breasts (10.0 per cent). These 10 contained 6 breasts with 2 cancer foci or more and 6 breasts with areas of high degree of epithelial proliferation. Five of these cancerous nodules were of the order of magnitude of a red bean to the tip of a forefinger, the remaining being microscopically small. Moreover, the area of high degree of epithelial proliferation (though not cancerous) was found in 6 breasts (6.0 per cent), 2 of which had 2 such areas.

The early cancer foci may be classified into the following 3 groups according to the site of origin:

1) Nos. 1 to 4: Malignant findings were obtained of epithelial proliferation which had occurred not only in the main duct immediately beneath the nipple or relatively large ducts but also in some terminal ducts and acini.

In No. 1, there was found cystic dilatation in numerous ducts large and small, the epithelial cells of which made so active an intraductal sessile proliferation as to present a so-called cribriform pattern. Partly, papilloadenomatous epithelial proliferation and interstitial infiltration of connective tissue by proliferated epithelial cells were also noticed (Fig. 2).

In No. 2, irregular polygonal epithelial cells show, in dilated ducts near the nipple, highly papilloadenomatous proliferation and filled duct lumina; in some parts these proliferated cells made an invasive growth in the interstitial connective tissue and formed lumina of various sizes and shapes, thus presenting early features of adenomatous cancer. In addition, in the periphery of the breast (about 3 cm. apart from the above cancer focus) there was observed a solitary cancer focus presenting histologic pictures similar to those described above, and early epithelial proliferation was noticed also in some relatively dilated terminal ducts and acini surrounding the focus.

Fig. 2

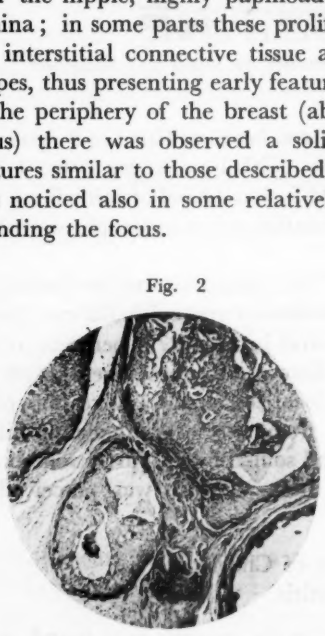

Fig. 3

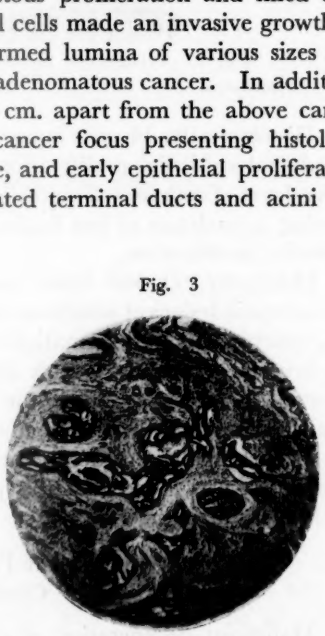

Fig. 2. Intraductal sessile proliferation of epithelial cells (so-called cribriform pattern) and infiltration of connective tissue by proliferated epithelial cells (No. 1), low magnification.

Fig. 3. Intraductal papillomatous epithelial proliferation and invasive growth of these proliferated epithelial cells (No. 3), low magnification.

In No. 3, the dilatation of ducts was not so remarkable as in Nos. 1 and 2, but there were seen papillomatous proliferation of epithelial cells

in many relatively large ducts and their surrounding branches and acini, and invasive growth of these proliferated epithelial cells in some parts (Fig. 3). Upon close examination of serial sections prepared from these parts there were found at least 4 isolated cancer foci together with a cancer focus, apart from them, presenting definite characteristic features of adenomatous cancer.

Thus, 2 to 5 solitary cancer foci were recognized in each of No. 2 and No. 3 cases, which may be taken to support the theory of multicentric development of cancer.

In No. 4, the main duct immediately beneath the nipple formed a cyst of a pigeon's egg in size; and part of its wall projected to form a tumor of the size of the tip of a thumb, which was histologically proved to be a papilloma with malignant degeneration. In this case, there was also found a papillomatous epithelial proliferation in other relatively large ducts and terminal ducts and acini.

2) Nos. 5 to 9: In terminal ducts and their acini there took place proliferation of epithelial cells, followed by malignant degeneration.

In Nos. 5 and 6, sessile proliferation of epithelial cells and high degree of epithelial spilling were seen in terminal ducts and their acini; these tubules and epithelial sprouts partly became dilated into a large number of small cysts (Fig. 4, A), and partly became disarranged to form irregular lumina and at some places to form solid epithelial cords, thus clearly presenting early features of malignant degeneration (Figs. 4, B, 5). In addition to these cancer foci, there were found in terminal ducts an area showing papillomatous (though not malignant) proliferation of epithelial cells.

Fig. 4

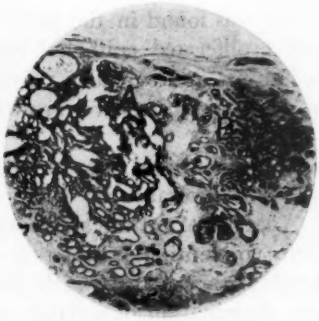

Fig. 5

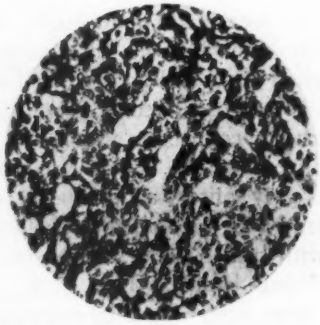

Fig. 4. Epithelial proliferation of terminal ducts and acini (A) and early malignant degeneration (B) (No. 5), low magnification.

Fig. 5. Early malignant degeneration (No. 6), high magnification.

Nos. 7 and 8 were found on both breasts of the same patient, similar

features as to malignant degeneration being shown in one area on the right side (No. 7) and in two areas on the left side (No. 8). In these cases there were seen epithelial proliferation in terminal ducts and acini too and infiltration of interstitial connective tissue by these proliferated cells (Fig. 6).

In No. 9, a definitely outlined colloid carcinoma, of the tip of the forefinger in size, was found apart from the nipple. This lesion is what had been perceived by touch as a well-defined migratory tumor before operation, which was also suggestive of fibroadenoma at first but was suspected of a cancer from a gelatinous appearance of its cut surface, and was microscopically confirmed to be a typical colloid carcinoma. In addition to

Fig. 6

Fig. 7

Fig. 6. Infiltration of connective tissue by epithelial cells proliferated in terminal ducts and acini (No. 8), medium magnification.

Fig. 7. Early malignant degeneration (A) arising from adenomatous proliferation and pale epithelium lining cyst (B) (No. 10), low magnification.

this, an area of early malignant degeneration was found in the peripheral region of the breast. In this cancer focus proliferated epithelial cells of terminal ducts and acini formed irregular lumina, and irregular polygonal cells formed solid cords or clumps, thus presenting invasive growth in interstitial connective tissue.

3) No. 10: No. 10 was a malignant degeneration from an acinus: Two early solitary cancer foci were found at 2 of several areas where acini presented an adenomatous proliferation. A large part of the adenomatous growth formed solid clumps or solid cords, presenting features of early carcinoma simplex (Figs. 7, 8). The development of these early cancer foci from acini was demonstrated by close examination of serial sections, for no proliferation of epithelial cells was observed in surrounding ducts.

As for the areas of high degree of epithelial proliferation in 6 breasts, there were found 2 cases of papilloadenomatous epithelial proliferation in the main duct or large ducts and their acini and 4 cases of epithelial pro-

liferation in terminal ducts and their acini (Fig. 9), were no malignant degeneration was noticed. In one of above mentioned cases, where epithelial proliferation was found in terminal ducts and acini, extraductal proliferation of the duct epithelium was observed in addition. According to Junge[5], the epithelium of the duct does not proliferate inward but outward, piercing the proper membrane and producing malignant degeneration there (extraductally). In my case, however, the proliferation did not go beyond expansive state toward malignancy (Fig. 10).

Fig. 8

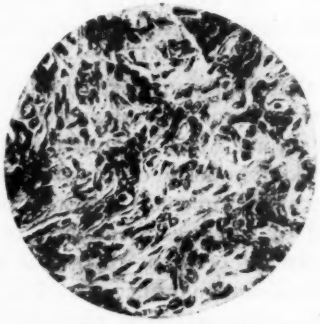

Fig. 9

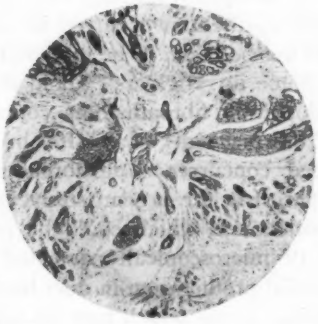

Fig. 8. High magnification of A in Fig. 7.

Fig. 9. High degree of epithelial proliferation in terminal ducts and acini, low magnification.

Fig. 10

Fig. 10. Extraductal expansive proliferation of epithelial cells of a duct, medium magnification.

The rate of occurrence of malignant degeneration was 18.5 per cent (5 breasts) of 27 breasts bearing macroscopic cysts, and 6.8 per cent (5 breasts) of 73 breasts without macroscopic cysts.

Malignant Degeneration of Pale Epithelium and of Fibroadenoma Concurrent with Chronic Cystic Mastitis

Though Kuru[1], and Higginson and McDonald[6] recognized malignant degeneration of pale epithelial cells, this does not seem to have prefectly established. In the present study, pale epithelium lining cysts occurred in 42 breasts (42.0 per cent) of the 100 breasts in chronic cystic mastitis. In 7 (16.7 per cent) of these 42, pale epithelium was found to show papillary formation, which was of a slight degree in all cases; in only one breast there was seen a moderate papillomatous proliferation of pale epithelium, but no malignant findings were perceived. One case where proliferation of pale epithelium occurred in connective tissue of hyaline degeneration could not be taken to be malignant.

Some investigators[1][5] hold the view that malignant degeneration will develop from fibroadenoma concurrent with chronic cystic mastitis. In 28 (28.0 per cent) of my 100 breasts in chronic cystic mastitis there was noticed concurrence of fibroadenoma. The number of fibroadenomas dealt with in the present study, however, was 43 in total, consisting of 24 fibroadenomas which could be perceived by touch as mentioned before and 19 microscopically detected ones. In 12 of them there was seen epithelial proliferation in duct lumina, but no malignant degeneration. In addition, a case which may be taken to be of fibroadenoma phyllodes was encountered in which connective tissue proliferated in intracanalicular fibroadenoma.

Histopathologic Features of Chronic Cystic Mastitis in Cancer Cases

In 8 (23.5 per cent) of 34 cases of mammary cancer there were noticed definite characteristic features of chronic cystic mastitis. The mammary cancer varied in size from the order of the tip of a forefinger to the order of a hen's egg. As for other changes in the breast than cancerous growths, various degree of connective tissue proliferation, epithelial proliferation, glandular tissue atrophy, duct and acinus dilatation, etc. were observed in all the cases. In addition, there were found pale epithelium in 5 of the 8 cases and fibroadenoma in 3. Furthermore, there were noted areas of epithelial proliferation in the tissue of chronic cystic mastitis in 4 of the 8 cases: a high degree of epithelial proliferation in terminal ducts and their acini in No. 13, a relatively high degree of papillomatous epithelial proliferation in Nos. 11, 14, and 16, and epithelial spilling concurrent with epithelial papillomatous proliferation in tubulus in No. 17.

In the tissue of the remaining 4 breasts there were seen duct lumina having atypical pale epithelial cells, but no clear findings characteristic

of chronic cystic mastitis were obtained. As plae epithelium lining cysts are seen in normal breasts (Frantz and others[7]) and fibroadenoma (Okamoto[8]), these 4 cases can not be taken to be of mammary cancer having its origin in chronic cystic mastitis.

The fact that 8 cases of mammary cancer gave also findings characteristic of chronic cystic mastitis may be taken to be suggestive of origination of the cancer in chronic cystic mastitis according to the general view that the latter runs a more chronic course than the former.

Rate of Occurrence of Malignant Degeneration

There is a large discrepancy among the reports thus far published concerning the rate of occurrence of malignant degeneration of chronic cystic mastitis; from 4 to 40 per cent, as shown in Table II. This may be

TABLE II

Rate of Occurrence of Malignant Degeneration

Author	No. of cases examined	No. of malignant examined	Rate of occurrence
Schimmelbusch[9]	43	3	7%
Tietze[10]			10%
Warren[11]	115	15	13%
Theile[12]	21	2	9%
Bloodgood[13]	31	2	10%
Fischer[14]	53	21	40%
Semb[15]	70	17	24%
Tokusige[16]	31	3	9.7%
Kuru[17]	60	9	15.0%
Soejima[18]	27	7	25.9%
Sato[4]	23	1	4.3%

due to differences in material examined as well as in the height of criteria for malignant degeneration; for example, the rate of the occurrence would become higher if cases in which histologic features of chronic cystic mastitis were observed in the mammary tissue of relatively advanced mammary cancer were included in cases of malignant degeneration of chronic cystic mastitis.

On the other hand, the rate of occurrence of chronic cystic mastitis in the mammary tissue of advanced mammary cancer differs much more largely from report to report: 3 per cent (Warren[11]), 14 per cent (Fischer[14]), 20 per cent (Cheatle and Cutler[19]), and 80 per cent (Semb[20]). Kuru[1] recognized histologic features of chronic cystic mastitis in a large

majority of mammary tissues bearing small cancer foci. Of 34 cases of mammary cancer in the present study, 8 cases (23.5 per cent) presented manifest features characteristic of chronic cystic mastitis.

The rate of occurrence of early cancer foci was 10.0 per cent in my study with 100 breasts in chronic cystic mastitis. However, if the cases in which high degree of epithelial proliferation was noticed are regarded as "precancerous" and included in cases of malignancy, then the rate of malignancy becomes 16.0 per cent (16 cases). Furthermore, if those 8 cases in which characteristic features of chronic cystic mastitis were seen in the breasts that had been diagnosed as mammary cancer are also added, the cases of chronic cystic mastitis become 108 in number, then the rate of malignancy becomes 16.7 per cent (18 cases); if a high degree of epithelial proliferation is also regarded as malignant, the rate becomes 22.2 per cent.

Summary and Conclusions

1. Diffuse fibrosis was observed in all of 100 breasts in chronic cystic mastritis, while macroscopic cyst formation occurred in only 27.0 per cent (27 breasts) of the breasts. Fibroadenoma (21 breasts, 24 nodules), a high degree of epithelial proliferation or early malignant degeneration (11 breasts, 13 nodules), and localized proliferation of connective tissue (5 breasts, 6 nodules) could be perceived by touch as nodules in diffuse fibrosis (in a total of 35 breasts, 35.0 per cent).

2. Early malignant degeneration occurred in 10 breasts (10.0 per cent) of the 100 breasts examined; 6 of these 10 breasts beared 2 cancer foci or more, 6 breasts of which had areas of high degree of epithelial proliferation. There were seen areas of high degree of epithelial proliferation in 6 breasts, 2 of which beared 2 such areas.

The sites of origin of early malignant degeneration or epithelial proliferation were, as reported in the literature, a) the main duct or relatively large ducts and their acini, and b) terminal ducts and their acini in many cases. With 1 case it was demonstrated by examining serial sections that 2 cancer foci had arisen each from an acinus.

As for the relation of malignant degeneration with the presence or absence of macroscopic cysts, the degeneration occurred in 18.5 per cent of the breasts bearing cysts, and in 6.8 per cent of the breasts without cysts.

3. Pale epithelium lining cysts occurred in 42 (42.0 per cent) of the 100 breasts. Though some extent of proliferation of pale epithelial cells was recongized in the cysts, canceration did not take place. Fibroadenoma was concurrent in 28 breasts (28.0 per cent) in cluding those microscopically detected; no malignant degeneration was noticed.

4. Manifest features characteristic of chronic cystic mastitis were

presented by 8 (23.5 per cent) of 34 cases of mammary cancer. These 8 have been regarded as those cases in which cancer originated in chronic cystic mastitis.

5. Early malignant degeneration was perceived in 10 (10.0 per cent) of the 100 breasts of chronic cystic mastitis. In addition to this, a high degree of epithelial proliferation occurred in 6 breasts. If this proliferation is taken to be a " precancerous " and those cases with such a proliferation are added to cases of malignant degeneration, the rate of occurrence of malignant degeneration is 16.0 per cent (16 breasts). Furthermore, if those 8 cases in which characteristic features of chronic cystic mastitis were seen in the breasts that had been diagnosed as mammary cancer are also added, the case of chronic cystic mastitis become 108 in number, 18 (16.7 per cent) of which showed malignant degeneration. If precancerous degeneration is taken to be malignant, the rate becomes 22.2 per cent (24 breasts).

I wish to express my sincerest thanks to Prof. K. Okamoto of the Pathological Department for his advice on the histological investigations.

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Noradrenaline in the Adrenals of Young Dogs

By

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(Received for publication, March 9, 1955)

So far as the noradrenaline content of the adrenal gland in young animals is concerned, only few evidences have been given (Shepherd and West,[1] Holton[2]). In cats, rabbits and guinea-pigs, it was found by Shepherd and West that the relative noradrenaline content of the adrenal gland in young animals was larger than that in adult ones. In dogs, however, such evidence was not obtained by their hands.

The present study was undertaken to know whether the positive result is obtainable or not concerning the adrenals of young dogs.

Methods and Results

Two series of experiments were made. First, 14 dogs of 2–7 days old were experimented on. And then, 13 dogs of 20–30 days old were used. In the former cases, the adrenal glands from a certain number of dogs were put together and in the latter the extracts were usually made from the adrenals of both sides in each dog except for one case (Exp. 5), in which the glands from 2 dogs were extracted together.

The glands were extracted with 4% trichloracetic acid. In the estimation of the noradrenaline and adrenaline contents in the extracts, our permanganate method[3] was relied on.

All the data are displayed in the accompanying Table.

In dogs of 2–7 days old, the noradrenaline and adrenaline contents per g. of adrenal gland were found to be 0.49 mg. (0.25–0.93 mg.) and 0.25 mg. (0.15–0.33 mg.), respectively. The relative amount of noradrenaline was 52–74%, the average being 64%. As to the dogs of 20–30 days old, noradrenaline and adrenaline contents per g. of gland weight were estimated as 0.35 mg. (0.14–0.66 mg.) and 0.47 mg. (0.26–0.63 mg.), respectively. The relative noradrenaline content was 47% (26–52%).

In my investigation previously reported,[4] it was found that the relative noradrenaline content in the adult dog was 18.5% (7–21%). Comparison of this figure with those of the present study may lead us to the conclusion

TABLE I

Noradrenaline Content in the Suprarenal Extracts of Young Dogs

No. of exp.	Age (days)	Numbers of animals	Numbers of glands	Body weight (kg.)	Weight of glands (mg.)	mg. per g. of gland			Percent noradrenaline
						Adrenaline	Noradrenaline	Total	
(A) Dogs of 2–7 days old									
1	2	3	6	1.2	161	0.25	0.27	0.52	52
2	5	4	8	1.3	226	0.15	0.25	0.40	63
3	7	1	2	0.4	60	0.33	0.93	1.26	74
4	7	6	12	2.4	393	0.28	0.49	0.77	64
	Mean					**0.25**	**0.49**	**0.74**	**64**
(B) Dogs of 20–30 days old									
5	20	2	4	2.5	363	0.26	0.15	0.41	37
6	20	1	2	1.1	144	0.60	0.66	1.26	52
7	26	1	2	1.4	258	0.38	0.29	0.67	43
8	26	1	2	1.2	249	0.47	0.31	0.78	40
9	26	1	2	1.8	274	0.45	0.28	0.73	38
10	26	1	2	1.8	318	0.48	0.32	0.80	40
11	30	1	2	1.6	268	0.40	0.14	0.54	26
12	30	1	2	1.6	275	0.43	0.36	0.79	46
13	30	1	2	1.3	218	0.36	0.28	0.64	44
14	30	1	2	1.6	279	0.60	0.35	0.95	37
15	30	1	2	1.5	226	0.63	0.65	1.28	51
16	30	1	2	1.2	166	0.53	0.45	0.98	46
	Mean					**0.47**	**0.35**	**0.82**	**42**

that the relative amount of noradrenaline of the adrenals in young dogs is definitely larger than that in adults.

SUMMARY

In dogs of 2–7 days old as well as those of 20–30 days old, the noradrenaline and adrenaline contents of the adrenals were estimated by means of the permanganate method.

It was found that the relative noradrenaline content of the adrenals in young dogs was invariably larger than that in adult dogs.

The auther wishes to acknowledge the helpful suggestions of Prof. T. Suzuki.

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Electrophoretic Study of Cerebrospinal Fluid Protein Components

Part 1. Survey in Normal Liquor

By

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The measurement of total protein content and the proportion of globulin in the cerebrospinal fluid (liquor) have long been used as a practical aid method for clinical diagnosis of various neurological diseases, especially in neurosyphilis. Recently the study by means of the electrophoretic method has been established, nevertheless, there are few data obtained from the liquor by this method. This might in part be due to the fact that the protein amount contained in the liquor is too small to make chemical analysis in detail.

By means of the Tiselius' electrophoresis,[1] the author has studied this problem for the past three years and recently has been able to reach the conclusion that the electrophoretic pattern of the concentrated liquor was almost similar to that of the serum. In this paper, the results from the cases in which each liquor was diagnosed by the routine clinical diagnostic method[2][3] are reported briefly.

Experimental

Methods and Subjects

During the procedure of pneumoencephalography, the liquor was obtained from each patient as large as 80 ml. This liquor, part of which was examined by routine clinical methods, was then dialysed, putting into a dialysing cellophane sac for about 24 hours against running water. Following this procedure, the liquor was immediately concentrated under diminished pressure to the extent of 10–15 mm. Hg at 20°C. This materials, then, was redialysed against a phosphate buffer at pH 7.8 and ionic strength of 0.145. The protein concentrations of these samples estimated by refractometer varied from 0.8% to 1.2% and the concentrated liquor was finally analysed by the Tiselius apparatus made by Hitachi Co.

In each case, the areas under enlarged curves of the descending boundaries taken from different angles were measured by a planimeter and the concentration of the liquor components on the average value was calculated in term of per cent against the total protein amount.

It is difficult to obtain a large dosis of liquor from the normal subjects, therefore, the author selected the liquor obtained from a series of the so-called " functional psychosis " having the following characters: no history of syphilis and alcohol-addiction, negative Wassermann' reaction of the liquor and the serum, negative Pandy and Nonne's reactions, normal colloidal reaction and especially normal pneumoencephologram.[5)6)] And, at the time of liquor puncture, these patients had of course been in normal condition in both somatic and psychotic spheres after clinical treatment.

Results

As shown in Table I, the total protein amount was estimated as high as 100 ml in mean value, ranging from 14 to 28 mgm. The mobility of each fraction and the concentration of protein components are shown in Fig. 1 and Table II. Concerning the mobility of the liquor, it might be able to say that the results correspond closely to the condition recognizable in the blood. Also, some cases showed to contain a small amount of the component designated as " X " which had such a mobility being faster than that of albumin, as shown in Fig. 1 (A, B, and D). Among those 19 cases studied, a clearly resolved component of anodic mobility being greater than albumin was recognized in 4 cases. The mean value of the X-component was 0.4 mgm. per 100 ml.

The percentage composition and the amount of each fraction in mg. 100 *ml.* are also shown in Table II. The albumin/globulin ratio in normal liquor was 1.2 in mean value. The relation between the normal globulin/albumin quotient and its related values are shown in Table III. In those cases, the mean value of globulin/albumin quotient was calculated at 0.83.

Comment

Kabat *et al.*, using a phosphate-sodium chloride buffer mixture at pH 7.4 and ionic strength of 0.2, reported the existence of the so-called fast component in 3% among 40 cases. Concerning the liquor obtained from 22 cases, though insufficiently concentrated, Scheids[9)] observed this fast component only in one case. Also, Fisk *et al.*,[10)] using a Klett Tiselius apparatus, reported almost same findings. Recently, Pieper,[11)] using the paper strip electrophoresis, recognized a rapidly migrating component in the normal liquor. Also as described above, we found a rapidly migrating component in the electrophoretic patterns of concentrated liquor equili-

TABLE I

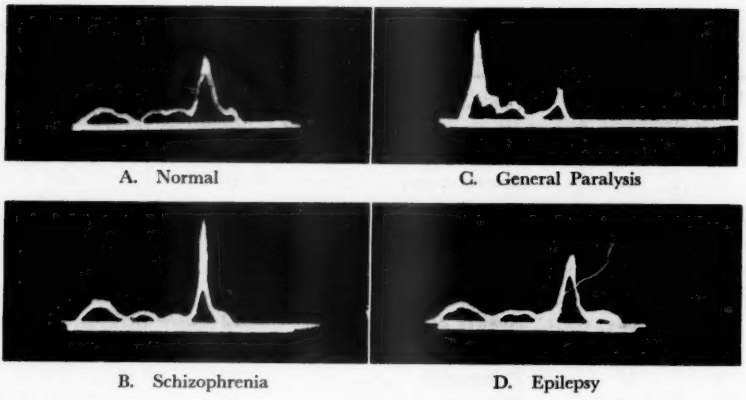
Total Protein in mg. per 100 ml.

Case No.	Age	Clinical Diagnosis	Total Protein mg%	Cell Count
1	50	Neurosis	27	2/3
2	24	Anxiety State	21	1/3
3	43	Neurosis	25	1/3
4	43	Neurosis	23	2/3
5	30	Neurosis	24	1/3
6	34	Neurosis	28	6/3
7	32	Anxiety State	22	5/3
8	19	Philoponism*	14	2/3
9	16	Philoponism	18	1/3
10	43	Philoponism	26	5/3
11	26	Philoponism	24	0/3
12	21	Philoponism	18	4/3
13	19	Philoponism	18	1/3
14	24	Neurosis	20	2/3
15	18	Philoponism	21	1/3
16	38	Neurosis	20	1/3
17	27	Neurosis	20	4/3
18	23	Philoponism	23	3/3
19	23	Philoponism	20	2/3

* Philoponism means psychosis due to wake-amine-intoxication.

brated with a phosphate buffer, though the value of components was comprised about 1.5% in the total protein.

Bücher *et al.*,[12] stated that the tau-fraction which possessed its position between beta-globulin and gamma-globulin, might be a specific fraction observable in the normal liquor. The existence of this tau-fraction was ascertained by Mies,[13] Gries *et al.*[14][15] Plückthum *et al.*[16] stated that this fraction was composed of beta-2- globulin, considering it as the same fraction of that found in the serum. Ewerbeck[17] describes this fraction as phi-fraction which shows a mobility resembling to that of gamma-globulin. However, we have thought this phi-fraction would be the gamma-1-fraction, because the mobility of gamma-1-fraction, obtained by the author, was almost similar to that of the phi-fraction, as shown in Fig. 1–C. Esser[18] states that the beta-globulin contained in the liquor has such a significant meaning as the so-called " Cerebrogener Anteil " which is specific to the liquor itself. However, we do not like to agree with his opinion, because, in our results, the beta-globulin was almost same as that of the serum in both quantity and quality, and, therefore, the beta-globulin would not be



A. Normal C. General Paralysis

B. Schizophrenia D. Epilepsy

Fig. 1. Electrophoretic patterns of the spinal fluid in phosphate buffer pH 7.8 and 1.0.145. Temperature of test, 20°C. Exposure after starting current, 52 minutes. Mobilities of the components in liquid A:

$$\text{X}: \frac{1.7\times\left(\frac{64\times 0.0082}{125}\right)\times 0.3}{3120\times 0.08} = 8.58\ \text{cm}^2\cdot\text{volt}^{-1}\cdot\text{sec}^{-1}\cdot 10^{-6}$$

$$\text{Alb.}: \frac{1.5\times\left(\frac{64\times 0.0082}{125}\right)\times 0.3}{3120\times 0.08} = 7.57\ \text{cm}^2\cdot\text{volt}^{-1}\cdot\text{sec}^{-1}\cdot 10^{-6}$$

$$\text{Alpha}: \frac{1.2\times\left(\frac{64\times 0.0082}{125}\right)\times 0.3}{3120\times 0.08} = 6.06\ \text{cm}^2\cdot\text{volt}^{-1}\cdot\text{sec}^{-1}\cdot 10^{-6}$$

$$\text{Beta}: \frac{0.8\times\left(\frac{64\times 0.0082}{125}\right)\times 0.3}{3120\times 0.08} = 4.04\ \text{cm}^2\cdot\text{volt}^{-1}\cdot\text{sec}^{-1}\cdot 10^{-6}$$

$$\text{Gamma}: \frac{0.1\times\left(\frac{64\times 0.0082}{125}\right)\times 0.3}{3120\times 0.08} = 0.51\ \text{cm}^2\cdot\text{volt}^{-1}\cdot\text{sec}^{-1}\cdot 10^{-6}$$

specific to the liquor.

In addition, concerning the mean value of gamma-globulin, it is interesting to compare our data with the European authors' because the value in our date were slightly higher than theirs.

Summary

Electrophoretic study of the protein components in the normal cerebrospinal fluid was reported. In 4 of 19 cases, a rapidly migrating component was observed. The fast components were comprised to about 1.5% in the total spinal fluid proteins. The percentage composition of the spinal fluid protein was as follows: X, 1.5; albumin, 52.8; alpha-albumin, 7.3;

TABLE II

Concentration of the Protein Components of Normal Cerebrospinal Fluid

Where two values are given, the values refer to the composition of alpha-1 and alpha-2 or beta- and beta-2 components.

Case No.	Per cent composition					Mgm./100 ml. (calculated)				
	X	A	Globulin			X	A	Globulin		
			α	β	γ			α	β	γ
1	1.4	47.0	8.5	17.6	25.5	0.4	12.7	2.3	4.8	6.8
2		54.0	7.1	14.5	24.4		11.3	1.5	3.0	5.1
3		49.0	8.6	17.4	25.0		12.3	2.2	4.3	6.2
4		56.0	7.9	17.9	18.2		12.9	1.8	4.1	4.2
5		48.0	9.4	17.8	24.8		11.5	2.3	4.2	5.9
6	2.7	46.2	9.2	18.0	23.8	0.8	12.9	2.6	5.0	6.7
7		53.0	7.3	15.4	24.3		11.7	1.6	3.4	5.3
8		56.0	5.0	18.0	21.0		7.8	0.7	2.5	3.0
9		53.0	5.6	17.3	24.1		9.5	1.0	3.1	4.4
10		55.0	9.4	10.8	24.7		14.3	2.4	2.8	6.4
11		57.0	9.8	15.1	18.1		13.7	2.3	3.6	4.4
12	1.2	48.0	8.5	17.3	25.1	0.2	8.6	1.5	3.2	4.5
13		56.0	8.7	10.0	25.3		10.1	1.6	1.8	4.5
14		55.0	6.0	14.2	24.7		11.0	1.2	2.8	4.9
15		56.0	6.1	16.0	21.9		11.8	1.3	3.3	4.6
16		49.0	8.6	17.8	24.6		9.8	1.7	3.6	4.9
17	0.8	54.0	6.4	13.6	25.2	0.2	10.8	1.3	2.7	5.0
18		56.5	3.2	17.7	22.6		13.0	0.7	4.1	5.2
19		55.4	3.7	15.5	25.4		11.1	0.7	3.1	5.1
Mean	1.	52.8	7.3	15.8	23.6	0.4	11.4	1.6	3.4	5.1

beta-globulin, 15.9; and gamma-globulin, 23.6. The albumin/globulin quotient in the normal cerebrospinal fluid was 1.2 in meaned value.

For the technical assistance, the author wishes to express his gratitude to Messrs. Maki and Hakomori of the Medico-chemical Institute, Tohoku University.

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TABLE III

Relation between Albumin and Globulin in the Normal Cerebrospinal Fluid

Case No.	Globulin / Albumin	Alpha-Glob. / Albumin %	Beta-Glob. / Albumin %	Gamma-Glob. / Albumin %	Albumin / Globulin
1	1.09	17.5	36.3	52.6	0.91
2	0.85	13.1	26.8	45.1	1.17
3	1.01	17.5	35.5	51.0	0.99
4	0.78	14.1	31.1	32.5	1.27
5	1.07	19.5	37.0	51.6	0.92
6	1.10	13.7	29.0	45.8	0.90
7	0.88	18.8	36.7	47.2	1.13
8	0.79	8.9	33.4	37.4	1.25
9	0.89	10.5	32.6	45.5	1.11
10	0.81	17.0	19.6	44.9	1.23
11	0.75	17.1	26.4	31.8	1.33
12	1.06	17.0	37.2	51.0	0.93
13	0.78	15.5	17.8	45.1	1.27
14	0.80	10.9	25.8	44.9	1.23
15	0.77	10.9	28.5	39.1	1.28
16	1.04	17.0	36.3	50.2	0.96
17	0.83	11.7	24.8	45.9	1.20
18	0.76	5.6	31.3	40.0	1.30
19	0.80	6.6	27.9	45.8	1.24
Mean	0.83	13.8	30.2	45.5	1.20

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The Influence of X-Ray on Experimental Pneumoconiosis

I, On the Development of Fibrogic Nodules Produced by Inhalation of Quartz Dust

By

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(Received for publication, March 18, 1955)

Introduction

Although, inhalation of quartz dust is the prime cause in the pathogenesis of silicosis, it is also believed that the condition of lung tissue susceptible to the impingement of quartz dust is of some importance for the development of the disease.

For this reason, the author has studied the influence of factors other than dust inhalation itself, and had an opportunity of finding a marked development of fibrotic nodules in the quartz dust inhaled lungs successively exposed to X-ray irradiation.

It is generally known that the lung tissue heavily irradiated with X-ray sometimes shows fibrosis clinically, so that it does not appear unreasonable that an irradiation would influence its susceptibility to silicosis in similar way, but my experiments showed the influence to be beyond any past anticipations.

To verify my occasional findings mentioned above, I have made some systematic experiments on the subject, of which I will report the results hereunder.

Experimental

Methods

The experimental animals consisted of 72 rats including both male and female, aged about 3 months and about 180 g. in average body weight. They were divided into 3 groups of 24 each and subjected to the 3 modes of experiments as follows, respectively.

Groups A and B were made to inhale quartz dust in the same dust

Detailed Histological Findings on the Photograph.

Fig. 1. Hx. E. × 100

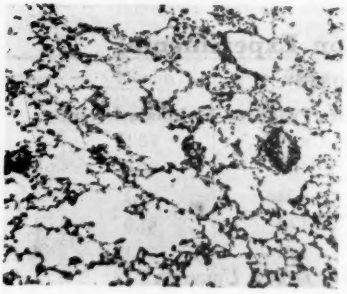


Fig. 2. Hx. E. × 100

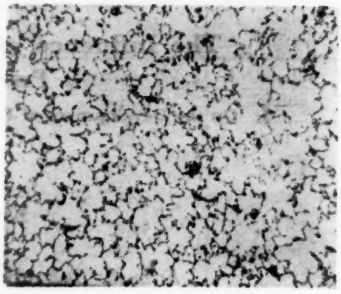


Fig. 1. Rat lung 60 days of survival term, 39 days of inhalation, 6 times of irradiation of X-ray : showing some slight granulosm.

Fig. 2. Control rat lung against (Fig. 1) rat : showing almost normal lung appearance.

Fig. 3. Hx. H. × 100

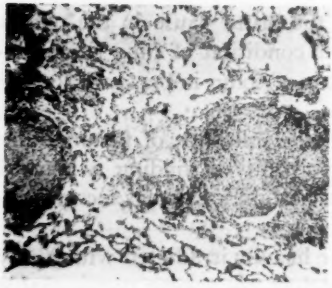


Fig. 4. Hx. E. × 100.

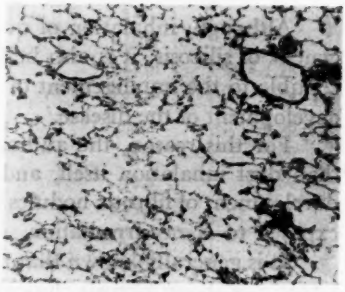


Fig. 3. Rat lung 175 days of survival term, 67 days of inhalation, 8 times of irradiation : showing one large nodule with coarse, and compact reticulin and a few collagen fiber (Grade 2 fibrosis).

Fig. 4. Control rat against (Fig. 3), survival 175 days, inhalation 67 days ; showing some small granulosm.

cabinet, and Groups A and C were X-ray irradiated under the samecon-ditions.

1) Method of inhalation : Quartz of Japanese product, containing 95.5% free crystal silica, as determined by N.S. Talvite's method, was pulverized in a ball mill and the dust dried in a sterilizer kept at 120°C immediately before use. The animals were exposed to a high concentration of this dust of 11,000 to 14,000 particles per cc. of air. 80% of the particles were below 3 micron in diameter, particles over 5 microns being only rarely contained. The dust blow was given for three hours each on three days every week.

Fig. 5. Hx. E.×100

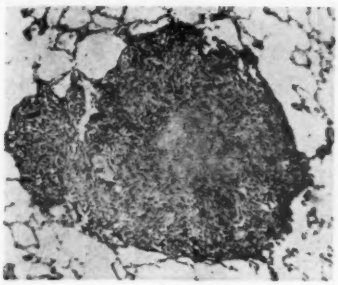


Fig. 6. Hx. E.×100

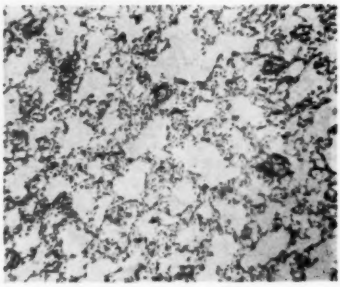


Fig. 5. Rat lung 197 days of survival, 84 days of inhalation, 14 times of irradiation: showing one large nodule with compact reticulin and almost collagen fiber (Grade 3,4).

Fig. 6. Control rat against (Fig. 5.), survival 197 days, 84 days of inhalation: showing somewhat large granulosm with slight reticulin fiber.

Fig. 7. Hx. E.×100.

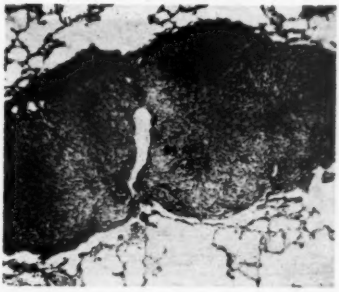


Fig. 8. Hx. E.×100.

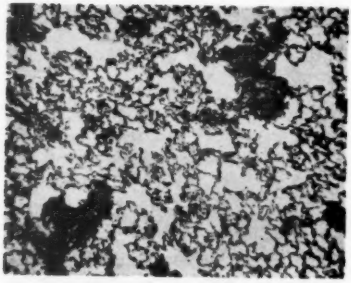


Fig. 7. Rat lung 260 days of survival term, 87 days of inhalation, 15 times of irradiation: showing many great and confluent fibrosis with compact reticulin fiber and collagenous fiber. (Grade 3.4)

Fig. 8. Control rat against (Fig. 7) 260 days survival, 87 days of inhalation: showing many granulosm and somewhat large silicotic granulosm with slight reticulin fiber.

2) Conditions of X-ray irradiation: A roentgen tube of 140 KVP was used under a tube current of 3 mA and with a 0.3 mm. Cu and a 0.5 mm. Al filters, giving a dose-rate of 7.1 r/min. at a distance of 50 cm. The animals in Groups A and C were given 30 r each on the whole body once a week.

3) Pathological technique: Routine postmortem examinations were carried out when an animal died or was sacrificed. The lungs of a killed animal were gently distended by injectiong abuot 10 *ml.* of 10% formol saline through the trachea which was exposed at the neck. Most of the

Fig. 9. Hx. E. × 100.

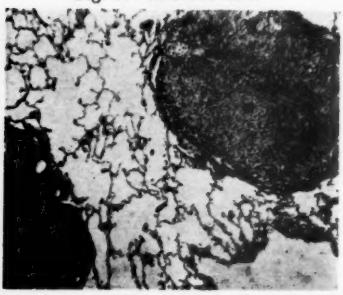


Fig. 10. Hx. E. × 100.

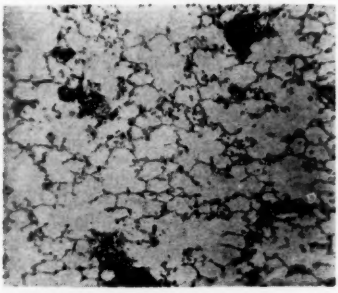


Fig. 9. Rat lung 267 days of survival term, 87 days of inhalation, 16 times of irradiation : showing many great nodule and confluent fibrosis with compact and collagenous fiber. Grade (3.4.)

Fig. 10. Control rat against (Fig. 9) 267 days survival, 87 days of inhalation : showing many granulosm and somewhat large granulosm with slight reticulin fiber.

Fig. 11. Mallory-Azan. × 100.

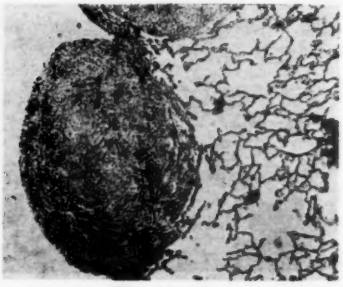


Fig. 12. Hx. E. × 100.

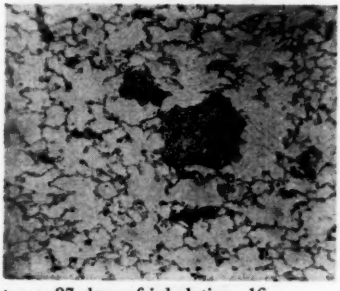


Fig. 11. Rat lung 267 days of survival term, 87 days of inhalation, 16 times of irradiation : showing many great nodule and confluent fibrosis with compact and collagenous fiber (Grade 3. 4.)

Fig. 12. Rat lung 366 days of survival, 84 days of inhalation quartz only treated : showing, somewhat great nodule with compact reticulin fiber and slight collagenous fiber.

air in the lungs was driven out by a gentle pressure on the sides of the thorax. The trachea was ligatured, the thoracic cavity was opened and the lungs, together with the subligated portion of the trachea, were taken out and fixed in formol. The lungs of the animals that died during the experimentation were first removed intact and then gently distended to the normal size, as satisfactory specimens could not be obtained otherwise. After for four days blocks were selected in the usual manner, cut into sections and stained with hamatoxylin-eosin and Mallory-Azan or Bielschwsky stains.

Gross Appearance of the Lungs.

a b

Fig. 13. a: Rat lungs, control to b rat, 175 days of survival term, 67 days of inhalation quartz only.

b: Rat lungs 175 days of survival term, 67 days of inhalation, 8 times of irradiation.

a b

Fig. 14. a: Rat lungs, control to b rat, 197 days of survival, 84 days of inhalation quartz only.

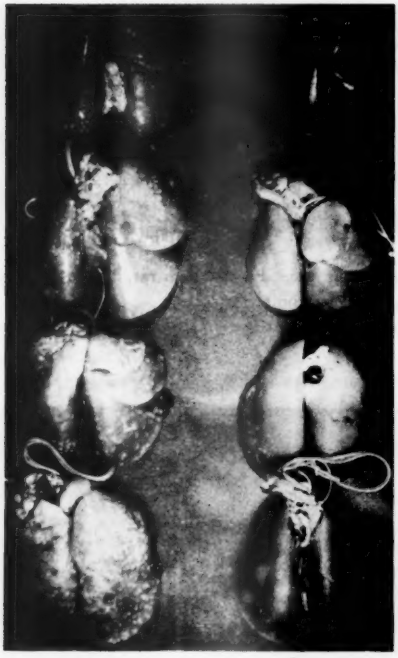
b: Rat lungs 197 days of survival term, 84 days of inhalation, 14 times of irradiation.

a b

Fig. 15. a: Rat lungs 366 days of survival term, 84 days of inhalation quartz only.

b: Rat lungs 267 days of survival term, 84 days of inhalation, 16 times of irradiation.

Gross Appearance of the Lungs (2)



a b

Fig. 16. a: Rats lungs in the A group; inhales quartz dust over five months, irradiated with 8 times or 15 times.
b: Rats lungs in the B group; inhaled quartz dust only over five months.

Results

The pathological changes produced in the lungs of the animals have been assessed according to Belt and King's classification. The five following grades of fibrosis were assumed: Grade 1, loose reticulin fibres with or without collagen formation; Grade 2, compact reticulin fibres with or without collagen formation; Grade 3, slightly cellular but almost collagenous; Grade 4, thoroughly collagenous and cellular, and Grade 5, acellular, collagenous and confluent.

1) Macroscopic findings of the lungs; In the first four months, the lungs and the pleura of the animals in all the groups shows little difference but in the fifth or sixth month, a great change came on in the lungs of every rat in the Group A, consisting in many nodular grayish-white spots of about 1 to 2 mm. in diameter on the surface of the lungs, mainly on the pos-

terior side. The lungs of these animals were also larger in general than of those in Group B.

In seven and eight months, these lesions observed in the Group A reached the maximum severity, but no evident change was found in any organ outside the lungs.

On the other hand, in the animals in the Groups B and C scarcely any change was obvious on the surface of the lungs.

2) Microscopic findings: By two months, marked changes had been produced in the lungs of the three sacrificed animals of the Group A, where multiple granulomata were recognized without individual reference, while in animals belonging to B, only one had a few granulomata in localized areas of its lungs.

In five months when other three animals of the each group were sacrificed, in the Group A, exposed to quartz dust on 84 days and to X-ray irradiation 14 times during the five months, the progress of silicotic fibrosis was found to have been very rapid indeed. Their lungs showed always slight or almost collagenous lesions of diffuse character (Grade 3) extending over wide areas, but the tracheobronichal lymph nodes were not yet very firm nor enlarged.

On the other hand, in the animals belonging to the Group B, we found only small fibrotic nodules of lower grade in some local fields of their lungs.

By the eighth or ninth month, four animals in the Group A subjected to more than 15 rounds of irradiation showed much more advanced fibrotic changes, severe fibrosis being found diffused all over the lungs in all cases with fibrotic nodules turned almost entirely collagenous (Grade 4).

In the Group B, made to inhale dust in the same manner as the Group A but not given X-ray irradiation, there were some fibrotic changes usually expected in such cases but no symptoms of packed reticulin fibres in their lungs, while in the Group C exposed to 15 rounds of X-ray irradiation but to no dust inhalation, no fibrotic change was ever found in their lungs.

3) Microscopic findings of other organs: No notable change was observable in the livers, the spleens and the kindneys of any animal within the eight months under observation, and there was no conspicuous change in the blood picture either.

Discussion

By these experiments, I could ascertain that silicotic nodules were clearly developed in the Group A of animals exposed both to quartz dust inhalation and X-ray irradiation within 5 or 6 months, while in the Group C subjected only to X-ray irradiation, no obvious change in the pulmonary tissue was observed even in the tenth month. The animals in the Group A showed clear indication of fibrotic nodules within two months already,

and the development of such nodules in this group was so rapid that the Groups A and B became much differentiated in their silicotic changes thereafter.

Thus I succeeded in establishing my occasional finding of an increased and accelerated formation of silicotic nodules in the lungs of rats exposed to quartz dust inhalation and to X-ray irradiation to be not a mere occasional finding but an observation of a constant actuality.

It is generally known that X-ray irradiation acts in stimulating increased preduction of connective tissue, but clinically the increase is substantiated when the dose of irradiation is large enough, small doses hardly showing any appreciable effect.

In experimental works, however, some apparent increase of connective tissue cells following a small dosis of X-ray irradiation upon experimental irradiation have been reported by Hirose, Serizawa and Taguchi.

When heavy irradiation is administered, some fibrotic changes, such as cancers of the lungs and the breasts, have been reported to develop in the lung tissues, but when the dosis of the X-ray irradiation is small, for example, as in X-ray therapy of pulmonary tuberculosis, increase of fibrotic changes, though anticipated, have not been confirmed.

Nevertheless, it is quite rational to presume that the lung tissue should respond against a slight irradiation in much the same manner, at least in principle though not in degree, as against a heavy one.

Thus, it may be said that the strong response of the quartz dusted and X-ray irradiated lungs of rats represents a phase of the general rule of roentgen induced fibrosis, only somewhat accentuated by the combination of fibrotic tendencies induced by repeated quartz inhalation.

It is, so to speak, a misfortune that in silicosis the increase of the connective tissue means a progress of the disease, while in many other diseases, the same process means rather a cure.

Conclusion

1. Experimental silicosis was rapidly and intensively developed by quartz inhalation associated with simultaneous X-ray irradiation.

2. Mere quartz dust inhalation without accompanying X-ray irradiation induces silicotic nodules only slowly and moderately, in comparison with the preceding.

3. In animals exposed to X-ray irradiation only and to no dust inhalation, no evident changes were detected in the lungs within nine or ten months.

4. From these results mentioned, we might conclude that the X-ray, when applied to silicotic lungs, emphasizes the progress of the disease.

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On the Minimum Effective Dose of Noradrenaline for Causing the Increase of the Blood Sugar Content in Non-Anesthetized Dogs

By

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(Received for publication, March 24, 1955)

It is generally accepted that the glycemic action of noradrenaline is weaker than that of adrenaline.[1)] However, there has been no investigation, in which the minimum effective dose of noradrenaline for causing the increase of the blood sugar concentration in non-anesthetized dogs was estimated. The present investigation was carried out in order that this dose might be determined.

Method

Thirty experiments were performed on 27 normal adult dogs without narcotizing or fastening. Each animal, not fed since the previous afternoon, came into use. Under local anesthesia with 1% procaine solution, the saphenous vein was prepared for the injection of noradrenaline and the external jugular vein for the collection of blood samples.

In the present investigation the noradrenaline solution was given intravenously at a uniform rate for 10 minutes with the special apparatus constructed of a 10 m*l*. syringe and a metalic screw. The solution of noradrenaline for injection was prepared just before use by diluting a stock solution which had been made by dissolving synthetic DL-noradrenaline base (Sankyo Co.) with 1/10 *N* HCl as a 1:1,000 dilution.

Experimets were performed under as nearly physiological conditions as possible.

Blood samples were taken immediately before and every 10 minutes after injection of noradrenaline. Puls rate, respiratory rate and rectal temperature were also measured. Blood sugar content was estimated by Hagedorn-Jensen.

Results and Discussion

The results are given in Table I.

Table I

Effects of Various Doses of DL-Noradrenaline on the Blood Sugar Content in Non-Fastened, Non-Anesthetized Dogs

No. and sex of dog	Body weight (kg.)	Amount of DL-noradrenaline injected per kg. per min. (μg.)	Blood sugar content (mg./dl.)							Heart beat (per min.)		Respiration (per min.)		Rectal temperature (°C)	Room temperature (°C)
			Before injection	After finishing injection (min.)						Before injection	After injection	Before injection	After injection		
				0	10	20	30	40	50	60						
5♀	7.2	3	111	112	114	109	107	107	105	107	145	142–180	36	44– 76	36.9–37.2	9–9.5
12♂	10.1	3	106		110	108	108	106	106	108	106	84–108	28	26– 68	38.1–38.6	19.5
1♀	6.4	5	115	113	117	119	114			119	122	72– 98	38	18– 28	38.2–38.5	16–16.5
2♂	10.2	5	119	120	122	119	122	124		119	116	64– 90	32	34– 68	38.6–38.8	14.5–15
4♀	9.2	5	120	122	120	119	117	115	113	115	136	110–140	32	25– 38	38.2–39.3	18
8♀	7.8	5	95		97	95	97	95	93	92	120	94–122	19	18– 52	39.0–39.2	13–14
15♂	12.4	5	93	99	96	90	93	95		97	82	58– 90	16	20– 48	38.0–38.5	22.5
26♀	9.0	5	97	97	102	95	95	92	92	92	110	92–130	32	26– 40	37.0–38.0	20.5–21
5♀	7.2	10	115	117	115	113	110	111	113	115	156	160–212	40	40–108	37.0–37.1	17–18
6♂	12.1	10	101	106	104	102	101	101	97	99	120	100–124	34	28– 60	38.2–38.6	15.5
11♀	9.7	10	108		125	124	113	110	106	104	92	80– 88	30	22– 36	38.4–38.8	20
14♂	11.5	10	97		104	104	95	93	93	95	124	110–136	18	26– 34	38.1–38.4	18.5
16♂	16.7	10	111	113	117	111	111	109	111	112	82	68– 98	22	20– 46	39.0–39.2	24
19♂	11.5	10	113		111	110	108	111	108	106	140	112–140	88	110–126	39.6–39.4	30–30.5
21♂	26.5	10	102		104	104	106	104	93	93	80	58–180	20	67–178	38.0–38.2	23–23.5
24♀	14.7	10	101	134	125	120	119	108	106	104	86	92–140	48	20– 62	37.0–38.0	19–19.5
25♂	9.5	10	99	102	99	95	95	95	93	93	124	80–100	32	17– 24	38.1	20–20.5

| | | |

this dog from 119 to 145 mg./d*l*. As control experiments the diluted HCl solution was administered at a uniform rate for 10 minutes, but no fluctuation of blood sugar level was observed.

Summarizing these experimental results, it may be concluded that the minimum effective dose of DL-noradrenaline for causing the increase of the blood sugar content is 15 μg. per kg. per minute. Taking L-noradrenaline to be twice as active as DL-noradrenaline, the minimum effective dose of the former will be 7.5 μg. per kg. per minute.

As to the minimum effective dose of L-adrenaline for raising blood sugar level of non-anesthetized dogs, Ooisi's data[2] showed that it was 0.3 μg. per kg. per minute. Consequently, concerning the glycemic effect, the activity ratio of L-noradrenaline to L-adrenaline calculated from minimum effective doses may be 1:25 in non-anesthetized dogs.

On the other hand, the pressor effect of L-noradrenaline in non-anesthetized dogs is larger than that of L-adrenaline, which was elucidated by the experiments of Ninagawa[3] in our laboratory.

Bernsmeier, Wild & Giertz[4] stated that noradrenaline given in a dose from 0.1 to 0.4 μg. per kg. per minute intravenously for 5–15 minutes to human beings had a considerable effect on circulation system, whereas it had none on blood sugar, Kappert *et al.*[5] reported that a dose of noradrenaline, which caused a marked pressor effect, produced little subsequent change in the blood sugar level. These coincide with the results of the present study.

In the present investigation tachypnoea was commonly observed during and after injection. The fluctuation of body temperature was always small. Bradycardia was found in many cases, though there were a few cases in which tachycardia was observed.

Summary

In non-anesthetized and non-fastened dogs, DL-noradrenaline was injected intravenously in a dose varying from 3 μg. to 30 μg. per kg. of body weight per minute for 10 minutes at a uniform rate and blood sugar content before and after noradrenaline injection was estimated.

The minimum effective dose of DL-noradrenaline for causing the increase of blood sugar content was found to be 15 μg. per kg. per minute.

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Experimental Studies on Intermediate Metabolism in the Kidney in Hypertension

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In recent years, many studies have been made on the metabolism of various substances in hypertensive patients, and it has been established that the high blood pressure is accompanied by some deviations in the metabolism. The kidney, maintaining a functional correlation with the liver, plays a leading role in the metabolic process in individual organism (Yano,[1] Hecht,[2] Saiki,[3] Melchier,[4] Kashimura,[5] Shida,[6] Shioji[7]) and though the pathologic physiology of hypertension is exceedingly complicated, it is beyond doubt that the renal function is closely related with hypertension, whether as the cause or effect of the disease, so that if no functional disturbance is yet apparent, some histological findings may be demonstrated in the vascular system of the kidney in hypertensive patients (Fishberg,[8] Goldblatt,[9] Matsuoka[10] and others). With such a fact in mind, the author of this paper undertook the following experiments, for the purpose of examining how the kidney behaves in face of and what parts it plays in the disordered metabolic process observed in hypertensive disease.

Experimental

Method

Normal rabbits weighing about 2 kg. were used. The blood pressure was measured every day at a fixed hour of hunger at the central auricle artery, following Kuraya[11] and Sato's[12] method. The measurement was repeated more than 10 times at some half minute's intervals and the average of the measured values was taken as blood pressure value. For inducing hypertension in rabbits, the animal was kept fasting through the morning and 1.5 cc/kg of 25% ethyl urethane solution was subcutaneously injected. After it was kept at rest for a while, a blood sample was drawn from the femoral artery, then the rabbits was fixed in ventral position, the second

sample drawn from the left renal vein, and a clamp was affixed to the left main renal artery according to the method of Goldblatt,[13] again one week later to the right main renal artery in a similar manner. One week thereafter, another blood samples were taken from the femoral artery and the renal vein. The blood samples were always below 3 cc. per sampling, and taken as slowly as practicable, without any congestion. All the samples were subjected to the measurements described hereunder, to analyse the intermediate metabolism of water, proteins, carbohydrates and electrolytes in the kidney through a comparative study of their contents in the arterial and venous bloods. The experiments were performed both with normal rabbits and Goldblatt's hypertensive rabbits.

The method of estimations were as follows:

For hemoglobin, Fleischl-Mischer's hemoglobinometer; for total serum and plasma protein, Pulfrich's immerse refractometer; for colloidal osmotic pressure (C.O.P.), Krogh and Nakazawa's method;[14] for plasma protein fraction, Tiselius's electrophoresis; for non-protein N, micro-Kjeldal's method; for serum NaCl, Schales and Schales's method;[15] for blood sugar, Hagedorn—Jensen's method; for blood lactic acid, Anrep and Cannan's method modified by Inawashiro and Hayasaka;[16] for plasma electrolytes (Na, K and Ca), flame spectrophotometer and for plasma pH, Beckman's glass electode pH meter.

Results and Discussion

Estimations on normal rabbits

In the first series of experiments, on 21 normal rabbits the concentration of hemoglobin, protein and NaCl both in the arterial and renal venous blood were compared each other. The change in water content of the blood coming through the kidney was estimated by inference from the change of the concentration of hemoglobin. The mean values of the measurements are shown in Table I. In the next, 8 normal rabbits were examined on the change in plasma protein fractions in the blood running out from the kidney. The mean values are shown in Table II. In the third series, 10 normal rabbits were subjected to the comparative estimations of glucose and lactic acid contents in the renal venous and arterial bloods, as are shown in Table III. In the fourth series, the changes in Na, K, Ca and Cl in the blood occurring during the circulation of blood through the kidney were estimated in 8 normal rabbits. In this series also the change in pH of plasma in the kidney was measured. The mean values are shown in Table IV.

Estimations on hypertensive rabbits

Similar investigations were made with Goldblatt's hypertensive rabbits

TABLE I

Average Values of Hemoglobin, Serum Protein and Serum NaCl in 21 Normal Rabbits

Blood pressure mm. Hg	Hemoglobin			Serum protein			C.O.P.			C.O.P. per protein %			Non-protein N			Serum NaCl		
	g/dl			g/dl			mm.H_2O			mm.H_2O			mg/dl			mg/dl		
	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$
70	15.30	16.04	+4.8	6.24	5.93	−5.0	285	264	−7.4	45.8	44.4	−3.1	37.88	35.34	−6.7	570.2	543.2	−4.7

A: A. femoralis, V: V. renalis.

TABLE II

Average Values of Plasma Protein Fractions in 8 Normal Rabbits

Blood pressure mm. Hg	Plasma protein			Albumin			α-Globulin			β-Globulin			Fibrinogen			γ-Globulin			$\frac{A}{G}$		
	g/dl			g/dl			g/dl			g/dl			g/dl			g/dl			g/dl		
	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$
76	6.50	6.24	−4.0	4.01	3.69	−8.0	0.53	0.56	+5.7	0.70	0.73	+4.3	0.64	0.65	+1.6	0.60	0.61	+1.7	2.19	1.94	−11.4

A: A. femoralis, V: V. renalis.

TABLE III

Average Values of Blood Carbohydrates in 10 Normal Rabbits

Blood pressure mm. Hg	Blood sugar			Lactic acid		
	mg/dl		$\frac{V-A}{A}\times 100$	mg/dl		$\frac{V-A}{A}\times 100$
	A	V		A	V	
75	143	140	−2.1	27.61	26.44	−4.2

TABLE IV

Average Values of Blood Electrolytes in 8 Normal Rabbits

Blood pressure mm.Hg	Cl			Na			K			Ca			pH		
	mEq/l		$\frac{V-A}{A}\times 100$	mEq/l		$\frac{V-A}{A}\times 100$	mEq/l		$\frac{V-A}{A}\times 100$	mEq/l		$\frac{V-A}{A}\times 100$			$\frac{V-A}{A}\times 100$
	A	V		A	V		A	V		A	V		A	V	
76	98.5	95.8	−2.7	139.7	136.5	−2.3	3.17	3.16	−0.3	4.07	4.00	−1.7	7.48	7.53	+0.7

A : A. femoralis, V : V. renalis.

as subject, the values in the renal vein and the femoral artery of the water and proteins (Tables V and VI), carbohydrates (Table VII) and plasma electrolytes (Table VIII) being estimated. These values were then studied in comparison with the corresponding values of normal rabbits (Tables I–IV), and some interesting facts were derived thereout on the status of intrarenal metabolism in hypertensive rabbits.

In normal rabbits, the hemoglobin concentration of the blood in the renal vein is a little higher than that in the arterial blood. This is due to the partial release of the blood's water content into the kidney during the intrarenal circulation for excretion in urine, causing a slight condensation of the blood before reaching the vein (cf. Shida,[6] Shioji[7]). In the present investigation the hemoglobin concentration in the arterial blood is lower in hypertensive than in normal rabbits, the mean values being 13.66 g/dl : 15.30 g/dl, apparently partly due to the Goldblatt's operation of the kidney itself as described above. The increase of the hemoglobin concentration in the venous blood is a little less in hypertensive rabbits (4.1%) than in normal rabbits (4.8%), due to the weakened condensation of blood in the kidney in the former, pointing out slightly reduced release of water from blood in the kidney in hypertensive animal. Schroeder[17] has demonstrated that in Goldblatt's rabbits the function of excreting water and salt undergoes some disorder. It is suggested that there is a close relation between the occurence of such changes and the Goldblatt's operative con-

TABLE V

Hemoglobin, Serum Protein and Serum NaCl in Hypertensive Rabbits

Case	Body weight kg.	Systolic pressure mm.Hg	Hemoglobin g/dl			Serum protein g/dl			C.O.P. mm.H_2O			C.O.P. per protein mm.H_2O			Non-protein N mg/dl			Serum NaCl mg/dl		
			A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$
1	3.4→3.2	78→ 95	13.30	13.56	+2.0	5.68	5.47	−3.1	302	270	−10.6	53.2	49.4	−7.1	48.82	47.81	− 2.1	621.3	613.2	−1.3
2	3.0→2.7	75→100	12.41	12.65	+1.9	5.55	5.47	−1.4	246	232	− 5.7	44.3	42.4	−4.3	64.86	59.77	− 7.8	581.2	580.1	−0.2
3	3.0→2.7	75→ 95	13.84	14.00	+1.5	5.90	5.25	−11.0	252	226	−10.3	42.7	40.0	−6.3	45.85	40.65	−11.3	603.1	598.0	−0.8
4	3.0→2.5	78→105	13.21	13.60	+3.0	5.68	5.47	−3.7	237	215	− 9.3	41.7	39.3	−5.7	185.80	181.14	− 2.5	621.7	619.7	−0.3
5	2.4→2.3	55→ 98	13.32	14.12	+6.0	5.72	5.23	−8.6	250	215	−14.0	43.7	41.1	−5.9	69.20	53.07	−23.3	620.2	617.2	−0.5
6	2.4→2.2	65→ 95	13.20	14.50	+2.3	6.12	5.90	−3.6	270	245	− 9.3	44.1	41.6	−5.7	52.37	48.41	− 7.6	532.3	514.8	−3.3
7	2.3→2.0	60→100	13.30	14.12	+6.2	6.55	5.90	−9.9	274	230	−16.1	41.8	39.0	−6.7	39.25	38.28	− 2.5	497.3	473.2	−4.8
8	2.5→2.1	75→110	14.10	14.80	+5.0	5.89	5.51	−6.5	263	232	−11.8	44.7	42.1	−5.8	50.64	48.87	− 3.5	590.9	573.2	−3.0
9	2.4→2.0	70→120	15.80	16.00	+1.3	5.90	5.47	−7.3	258	222	−14.0	43.7	40.6	−7.1	47.60	46.42	− 2.5	579.2	576.2	−0.5
10	1.5→1.2	70→115	14.14	14.82	+4.8	5.62	5.23	−6.9	254	224	−11.8	45.2	42.8	−5.3	86.75	84.40	− 2.7	630.8	622.7	−1.3
Average	2.6→2.3	70→103	13.66	14.22	+4.1	5.86	5.49	−6.3	261	231	−11.5	44.5	41.8	−6.1	69.11	64.88	− 6.1	587.8	578.8	−1.5

A : A. femoralis, V : V. renalis. Figures with arrow of body weight and systolic pressure indicate the values before and after Goldblatt operation.

TABLE

Plasma Protein Fractions in

Case	Body weight kg.	Systolic pressure mm.Hg	Plasma protein			Albumin			α-Globulin		
			g/dl		$\frac{V-A}{A}\times 100$	g/dl		$\frac{V-A}{A}\times 100$	g/dl		$\frac{V-A}{A}\times 100$
			A	V		A	V		A	V	
1	2.1→1.9	70→115	6.23	5.64	−9.5	3.52	3.08	−12.5	0.56	0.58	+ 3.6
2	2.5→2.2	80→125	5.90	5.79	−1.9	3.36	2.95	−12.2	0.53	0.60	+13.2
3	2.0→1.9	70→130	6.55	6.23	−4.9	3.66	3.41	− 6.8	0.64	0.75	+17.2
4	2.0→1.8	70→130	6.55	6.23	−4.9	4.13	3.85	− 6.8	0.49	0.54	+10.2
5	2.5→2.1	80→140	6.55	6.35	−3.1	3.85	3.22	−16.4	0.77	0.82	+ 6.5
Average	2.2→2.0	76→128	6.36	6.05	−4.9	3.70	3.30	−10.1	0.60	0.66	+10.0

A : A. femoralis, V : V. renalis. Figures with arrow of body weight and systolic

striction of renal artery, giving rise to remarkable changes in the renal hemodynamics by reducing the renal blood flow, which in turn causes reduction of the glomerular filtration rate and finally the decrease of urine excretion.

It has been ascertained in a great series of research that when there is a disturbance in the kidney function, the total serum protein shows reduction, which is mainly due to the decrease in proteins of minute molecules, such as albumin. In the present experiments also it has been demonstrated that in hypertensive rabbits the albumin content in arterial blood is less than in normal subjects, the former showing the mean value of 3.70 g/dl, while in the latter, the mean value stands at 4.01 g/dl. This reduction in albumin concentration may be attributed partly to the Goldblatt's operation itself. The colloidal osmotic pressure is reduced in hypertensive cases, being on an average 261 mm. H_2O, while in normal it is averaged to 285 mm. H_2O. This also may suggest the decrease of albumin in serum (Tables I and V). The estimation of the plasma protein fractions with Tiselius's electrophoresis gives further proof of this reduction in details (Tables II and VI).

In hypertensive rabbits, α- and β-globulin as well as fibrinogen are found increased over those in normal rabbits, while γ-globulin shows little difference. The increase in β-globulin is especially marked. In normal blood α-globulin is contained on an average in the rate of 0.53 g/dl and β-globulin in the rate of 0.70 g/dl, while in the hypertensive blood, the values are 0.60 g/dl and 0.73 g/dl respectively.

As for the intermediate metabolism of proteins in the kidney, we find that the normal kidney takes in proteins, particularly albumin, into it, decomposes a great part of it, together with a part of tissue proteins of the

VI

Hypertensive Rabbits

β-Globulin			Fibrinogen			γ-Globulin			$\frac{A}{G}$		
g/dl		$\frac{V-A}{A}\times 100$	g/dl		$\frac{V-A}{A}\times 100$	g/dl		$\frac{V-A}{A}\times 100$			$\frac{V-A}{A}\times 100$
A	V		A	V		A	V		A	V	
0.69	0.80	+ 8.7	0.66	0.69	+ 4.5	0.64	0.70	+ 9.4	1.86	1.48	−20.4
0.80	0.92	+15.0	0.67	0.76	+13.4	0.54	0.61	+13.0	1.80	1.38	−23.3
0.89	0.95	+ 6.7	0.52	0.52	± 0	0.53	0.62	+17.0	1.78	1.47	−17.4
0.59	0.62	+ 5.1	0.67	0.69	+ 3.0	0.66	0.74	+12.1	2.37	2.03	−14.3
0.68	0.91	+32.4	0.66	0.72	+ 9.1	0.57	0.66	+15.8	1.91	1.35	−29.3
0.73	0.84	+15.1	0.64	0.68	+ 6.3	0.59	0.67	+13.6	1.94	1.54	− 20.6

pressure indicate the values before and after Goldblatt operation.

TABLE VII

Blood Carbohydrates in Hypertensive Rabbits

Case	Body weight kg.	Systolic pressure mm.Hg	Blood sugar			Lactic acid		
			mg/dl		$\frac{V-A}{A}\times 100$	mg/dl		$\frac{V-A}{A}\times 100$
			A	V		A	V	
1	3.0→2.7	65→100	191	180	− 5.8	29.57	28.59	−3.3
2	3.0→2.5	65→115	198	182	− 8.1	32.79	31.75	−3.2
3	2.4→1.9	75→ 95	160	153	− 4.4	27.64	26.20	−5.2
4	2.9→2.7	78→120	210	185	−11.9	35.36	34.61	−2.1
5	2.5→2.1	64→110	225	202	−10.2	34.07	33.21	−2.5
6	2.9→2.8	80→115	198	180	− 9.1	31.50	30.60	−2.9
7	2.8→2.5	75→100	209	183	−12.4	39.86	39.15	−1.8
8	2.5→2.0	65→115	200	183	− 8.5	32.79	31.98	−2.5
9	3.4→2.7	75→105	182	167	− 8.2	31.50	30.86	−2.0
10	2.5→2.2	80→140	240	205	−14.6	44.56	44.11	−1.0
Average	2.8→2.4	74→112	201	182	− 9.5	33.96	33.11	−2.5

A : A. femoralis, V : V. renalis. Figures with arrow of body weight and systolic pressure indicate the values before and after Goldblatt operation.

kidney, into non-protein N and excretes them into urine, while on the other hand discharges larger moleculed proteins of globulin class into the venous blood, so that the osmotic pressure of blood is not markedly reduced, thus acting in readjusting the colloidal osmotic pressure of blood (cf. Shida,[6] Shioji[7]). From the comparison of protein contents in the arterial and the venous blood in normal animal, it is inferred that, as given in Table II, in

TABLE VIII

Blood Electrolytes in Hypertensive Rabbits

Case	Body weight kg.	Systolic pressure mm.Hg	Cl			Na			K			Ca			pH		
			mEq/l			mEq/l			mEq/l			mEq/l			mEq/l		
			A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$	A	V	$\frac{V-A}{A}\times 100$
1	2.1→2.0	70→115	119.0	118.2	−0.7	148.2	152.0	+2.5	3.46	3.31	−4.4	3.86	3.62	−6.2	7.50	7.68	+2.8
2	2.5→2.3	80→125	104.5	105.0	+0.5	143.1	148.7	+3.9	4.28	4.16	−2.8	3.46	3.40	−1.2	7.64	7.83	+2.5
3	2.0→1.8	70→130	92.5	92.7	+0.2	139.7	138.8	−0.6	4.64	4.50	−3.0	3.50	3.34	−4.6	7.43	7.63	+1.3
4	2.5→2.2	80→140	98.1	98.4	+0.3	142.3	142.5	+0.1	4.36	4.18	−4.1	4.06	3.84	−5.4	7.42	7.53	+1.5
5	2.0→1.9	80→140	104.4	105.0	+0.6	143.7	142.5	−0.8	4.21	4.05	−3.8	3.75	3.52	−3.5	7.44	7.49	+1.2
Average	2.2→2.0	76→130	103.7	103.9	+0.2	143.4	144.9	+1.0	4.19	4.04	−3.6	3.73	3.54	−5.1	7.49	7.63	+1.9

A: A. femoralis, V: V. renalis. Figures with arrow of body weight and systolic pressure indicate the values before and after Goldblatt operation.

the renal venous blood the albumin content is decreased by 8%, while α-, β- and γ-globulin are increased by 5.7%, 4.3% and 1.7% respectively, and that fibrinogen content also is augmented in the venous blood. Thus, proteins of globulin class, in the forms of α- and β-globulin, sometimes also of γ-globulin, particularly α-globulin are carried out from the kidney. In hypertensive rabbits the protein metabolism in the kidney is carried on more actively, a much larger quantity of proteins of globulin class being deported from the kidney, to make up for the increased release of albumin from the circulating blood into the kidney (Table VI). Thus, while on an average 10.1% of albumin is decreased in the renal venous blood, α-globulin is increased by 10.3%, β-globulin by 15.1% and γ-globulin by 13.6% on an average. Consequently, while in normal cases, in the venous blood of the kidney $\frac{A}{G}$ is decreased by 11.4%, in hypertensive subjects this decrease is significantly greater, being 20.6% in the mean. The exportation of fibrinogen into the renal vein also is slightly increased in hypertensive cases compared with normal rabbits (6.3% :1.6%). Lewis and Page,[18] Araki[19] and Kozasa[20] maintain that the increase of β-globulin in the blood is related with hypertension or vascular disease; it may be of deep interest in collation with these remarks that the release of β-globulin and γ-globulin from the kidney is augmented in Goldblatt's rabbits, as observed in the present experiments.

The non-protein nitrogen in arterial blood is somewhat increased in hypertensive animals (Tables I and V), the mean values being 69.11 mg/dl, while it is 37.88 mg/dl in normal animals. Its discharge into the kidney is slightly reduced (6.7% :6.1%), suggesting accumulation of non-protein nitrogen in the blood, though to a little extent. This is perhaps partly attributed to the release of albumin in a large amount into the kidney and the augmentation of non-protein nitrogen in the kidney due to the decompensation of the renal tissue caused by the ischemic constriction of main renal arteries.

As to carbohydrate metabolism, the author finds that the arterial blood sugar level is markedly elevated in Goldblatt's rabbits. But this hyperglycemia is obviously rather due to the stress of operation than to hypertension itself. The release of blood sugar in the kidney is augmented in hypertensive animals to 9.5% on an average, the mean increase in normal rabbits being 2.1%. This increase is partially for adjusting the hyperglycemia, but since the kidney has the proper function of taking in sugar from the circulating blood for decomposition and excreting a minimum part of the uptake into urine (Himwick, Koskoff and Nahum,[21] Hemingway and Phelps,[22] Rose,[23] Itakura,[24] Tokumitsu,[25] Shioji[7]), it might be suggested that this function is somewhat accentuated in Goldblatt's rabbits.

Lactic acid is also found increased in the arterial blood of Goldblatt's rabbits over that in normal rabbits (33.96 mg/dl :27.61 mg/dl in mean values) and this may be also due to hyperglycemia. An examination of its intrarenal metabolism reveals that its discharge into the kidney is under normal (4.2% :2.5%). That is to say, the function of the kidney to take up lactic acid from blood and to decompose it (Himwick,[21] Wesselkina,[26] Shioji[7]) is to some extent impaired in the ischemic kidney of the hypertensive rabbits, pointing out on diminished disposition of lactic acid in the kidney.

Finally, the intermediate metabolism of electrolytes in the kidney was investigated (Tables IV and VIII). The Cl and Na contents in arterial blood in Goldblatt's rabbits were found increased on an average to 103.7 mEq/l and 143.4 mEq/l from the normal values of 98.5 mEq/l and 139.7 mEq/l respectively. K was markedly increased (3.17 mEq/l : 4.19 mEq/l), but Ca decreased (4.07 mEq/l :3.75 mEq/l).

A review of intermediate metabolism in the kidney of these electrolytes has revealed that in normal animals Cl is discharged from the blood into the kidney during circulation, so that its content in the renal venous blood is decreased (2.7% in average), while in hypertensive reabbits, the Cl content in the venous blood is not always found diminished from that in the arterial blood, rather tending to an increase in the renal vein, in which the change of Cl amounts on an average to +0.2%. The same trend is found also in the Na content. In hypertensive animals the arteriovenous difference of Na shows +1.0%, while in normal animals it is decreased to −2.3%. Thus, it may be said that Cl and Na are transferred from circulating blood into the kidney in normal animals, but in hypertensive cases, this transfer is greatly reduced and frequently even an opposite transition from the kidney into the blood is observed. This change is especially marked with Na.

In an earlier series of the present experiments (Tables I and V), the author has estimated the NaCl content in serum with Schales and Schales's method[15] and found that in 21 normal rabbits the mean content was 570.2 mg/dl in the arterial blood and 543.2 mg/dl in the renal venous blood, so that the discharge of serum NaCl into the kidney amounted to 4.7% on an average, while in Goldblatt's animals its mean value of 10 cases were 587.8 mg/dl in the arterial blood and 578.8 mg/dl in the renal venous blood, the release into kidney being diminished to 1.5%. This marked decrease of the release into the kidney is also in agreement with the above stated fact.

K and Ca are released into the kidney from blood in normal cases, so that their contents in the blood coming out from the kidney are less than those in the arterial blood, the decrease being for K 0.3% and for

Ca 1.7% on an average. In hypertensive rabbits, this decrease is intensified, so that the values rise to −3.6% for K and to −5.1% for Ca on an average. The fact that in hypertensive rabbits the taking out of these electrolytes from the kidney is diminished, seems to be of great interest.

The plasma pH (Tables IV and VIII) showed a slight rise in hypertensive rabbits, on an average 7.48 in normal and 7.49 in hypertensive cases. In normal animals the blood pH shows slight rise of 0 7% on an average during circulation through the kidney, which is augmented in hypertensive rabbits to 1.9% on an average.

The kidney has the function to regulate the electrolyte contents in blood and to maintain the acid-base equilibrium. This occurs especially apparently in hypertensive animals, in particular, in Goldblatt's rabbits, in which the release of Cl and Na from the blood into the kidney is decreased, while that of K and Ca is increased, thus occasioning some changes in the intrarenal metabolism of electrolytes, obviously partly for the sake of neutralizing the acidosis caused by the accumulation of lactic acid in blood as above mentioned.

Summary

In order to investigate the intermediate metabolism of water, proteins, carbohydrates and electrolytes, in the kidney in experimental hypertension, the contents of these substances in the renal venous blood were estimated and compared with those in the arterial blood in normal rabbits as well as in the rabbits made hypertensive by the method of Goldblatt. The comparison of the corresponding data taken from both kinds of animals revealed following facts.

1. The release of water from the circulating blood into the kidney tends to be somewhat decreased in hypertensive animals.

2. The discharge of proteins from the blood into the kidney is augmented in hypertension. This increment is mainly due to albumin; on the contrary, globulin, especially β- and γ-globulin are transferred in a large quantity from the kidney into the circulating blood.

3. The discharge of blood non-protein nitrogen into the kidney tends to be reduced in hypertensive animals.

4. In hypertensive animals, the release of blood sugar into the kidney is augmented, while that of lactic acid is decreased.

5. Of the electrolytes in blood, the release of Cl and Na into the kidney is below normal; in some cases Cl and Na are transferred, though a little, in the reverse direction so that their contents in the renal vein are increased over those in arterial blood. The transfer of K and Ca into the kidney is increased over normal.

6. The rise of pH during the intrarenal circulation tends to be slightly augmented in hypertensive cases.

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On Epinephrine Test in Skin Diseases. I

By

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Introduction

Since Seyle has published his theory on the so-called " General Adaptation Syndrome ", the role of the pituitary-adrenocortical system in the defense mechanism of the body becomes important and attempts to inquire into the function of the pituitary-adrenocortical system following such reaction processes have been devised and proposed by many workers. As one of such methods, Thorn *et al.*, in consideration of the behaviour of eosinophil cells among the hematologic changes induced in alarm reaction in 1948, invented a test method for adrenocortical insufficiency using ACTH as reagent, the method now in wide use under the name of Thorn's test. Later on, Laragh *et al.* asserted that similar hematologic changes are elicited by administration of epinephrine and suggested that the puititary adrenocortical system plays a role in this reaction process, and Recant *et al.* have proposed the utility of using epinephrine in the place of ACTH in the test. This method is also being widely taken up by clinical practitioners. The hypothesis of the eosinopenic mechanism in this epinephrine test is built upon the supposition based upon animal experiments and clinical observations of cases including patients with adrenocortical insufficiency that a series of reaction forms—epinephrine→hypothalamus→anterior lobe of pituitary gland→ACTH→adrenal cortexc→corticoid→eosinopenia—is occasioned following epinephrine administration and if any disturbance raises in the circle of this series of reactions, no decrease of eosinophils appears (neurohumoral mechanism). This theory is supported also indirectly by the theories of self-regulation humoral mechanism and sympathico adrenal mechanism (Long *et al.*). However, whether the eosinopenic response is elicited by the above cited series of reactions only, or some other mechanism may enter the process or even quite a foreign mechanism may produce the same response is attended with many an unsolved problem, and this is the main reason why there are as yet dis-

agreements on the adequacy of this method. For example, Hitzelberger and Ruppel, Lomeyer, Kark, Waldenström, Okinaka, Hiraki and many others have expressed pessimistic views on the diagnostic value of this test from their standpoints of view. But these opponents of the theory have no decisive evidences to disprove the theory, and at present, we have to acknowledge its value as a tentative test method to indicate a probability of the pituitary-adrenocortical hypo-function. On the other hand, many supporters are also not lacking, including Biss *et al.*, Posey *et al.*, Goelker *et al.*, Heni *et al.* and Kresbach. Reports on application of this test to various diseases are also too numerous for enumeration. In the field of dermatology too, the test has been becoming a thema of active researches, as exemplified by the works of Lützenkirchen,[1] and in Japan, of Yoshimatsu,[2] Abe,[3] Ohmori[4] etc. According to several facts based upon some reported observations and a few findings of clinical investigations the author believed that if the method is properly applied with due consideration to certain conditions, it possesses the value as a screening test, if not as a decisive index, of the function of the pituitary-adrenocortical system, and have applied this test to 955 cases of skin diseases since 1952, with the aim of examining the relation between the skin diseases and the pituitary-adrenocortical function.

Tested subejcts and methods. The subjects of this study consisted of out- and in-patients of our Clinic. The standard dosis of epinephrine for subcutaneous injection was 0.3 cc. of 1:1,000 solution. For the technique, materials and conditions of the tests, the author followed the method originally established by Thorn *et al.* as far as possible.

Evaluation of the results. On the diurnal rhythms of circulating eosinophils, there are studies reported by Rud ($-20\%\sim+30\%$), Heni ($\pm 10\%$) and others. In adopting the standard for evaluating the results of the experiments, the author decided to follow the standard established by Posey *et al.*[5] in general, with reference to the above reports. That is to say, the results of 50% and above were estimated as normal (+), those between -49% and -30% as questionably abnormal ($\pm$) and those below -30% as abnormal ($-$).

Results of tests. As shown in the Table I.

Discussion

1) Allergic diseases. Thorn *et al.* have reported that, during the height of an allergic eosinophilia, the rate of reduction of eosinophils is often observed to decrease, owing to the enhanced production of these cells against peripheral destruction of eosinophils, and interpreted that this phenomenon should be called a false response in contradistinction from the reaction of a primary pituitary-adrenocortical deficiency. The

TABLE I

Diagnosis	No. of cases	Normal ～−50%	Questionably abnormal −49%～−30%	Abnormal −29%～	%of cases −49%～
Allergic diseases					
Eczema acutum	84	48	22	14	42.9
Eczema tyloticum rhagadiforme	14	8	5	1	42.8
Eczema chronicum	14	6	5	3	57.1
Neurodermitis circumscripta	8	7	1		12.5
Neurodermitis diffusa	15	10	4	1	33.3
Prurigo Besnier	8	5		3	37.5
Prurigo Hebra	7	6	1		14.3
Urticaria	42	24	7	11	42.9
Dietetic toxic eruption	14	8	2	4	42.8
Drugexanthema	13	9	1	3	30.7
Erythema exsudativum multiforme	14	9	4	1	35.7
Erythema annulare centrifugum	6	3	2	1	50.0
Erythematodes acute form	8	3	1	4	62.5
chronic form	19	11	6	2	42.2
Erythema nodosum	6	4	1	1	33.3
Purpura	11	6	2	3	45.4
Ectodermose érosive pluriorificielle	2	1	1		
Pruritus cutaneus	12	9	3		25.0
Pigmentary diseases					
Addison's disease	12	1	3	8	91.6
Riehl's melanosis	28	12	12	4	57.1
Pigmentatio postlésionnelle	13	8	2	3	38.5
Chloasma	51	37	6	8	27.5
Vitiligo	16	7	3	6	56.2
Vogt-Koyanagi, Harata's disease	2	1	1		
Nevus pigmentosus	11	9	2		18.2
Nevus fuscocaeruleus ophthalmo-maxillaris Ota	9	6	3		33.3
Nevus depigmentosus	2	2			
Epheliden	7	7			
Dyschromatosis symmetrica hereditaria	2	2			
Xeroderma pigmentosum	5	4		1	20.0
Ephelides inversa	2			2	
Seborrheic diseases					
Eczema seborrhoicum	50	38	7	5	24.0
Acne vulgaris	39	27	7	5	30.7
Rosacea	3	3			
Keratotic diseases					
Psoriasis	27	16	6	5	70.7
Parapsoriasis	4	2		2	50.0
Pityriasis rosea Gibert	7	4	2	1	42.9
Pityriasis rubra pilaris	6	4	2		33.3
Lichen ruber planus	8	3	3	2	62.5
Erythrodermia exfoliativa generalisata	12	3	7	2	75.0

Diagnosis	No. of cases	Normal ~−50%	Questionably abnormal −49%~−30%	Abnormal −29%~	%of cases −49%~
Ichthyosis	8	8			
Lichen pilaris	4	3	1		
Keratosis palmoplantaris hereditaria	4	2		2	50.0
Bullous disease					
Pemphigus	2		2		
Dermatitis herpetiformis	9	4	3	2	55.4
Hydroa vacciniformis	2		1	1	
Epidermolysis bullosa hereditaria	5	4	1		
Endocrine diseases					
Basedow's disease	1	1			
Myxedema circumscripta	1			1	
Mongolism	1			1	
Simmond's disease	2		1	1	
Diabetes insipidus	1		1		
Cushing's disease	1	1			
Acromegalia	1	1			
Diabetes mellitus	2	1	1		
Hair diseases					
Alopecia areata	102	42	33	27	58.8
Other alopecia	13	10	2	1	23.0
Hypotrichosis congenita	6	4	2		33.3
Hypertrichosis congenita	2	1	1		
Skin tumors					
Carcinoma cutis	2	1	1		
Reticulosarcoma cutis	1	1			
Hodgkin's disease	4		2	2	
Mycosis fungoides	2	1	1		
Acanthosis nigricans	4	3		1	
Keloid	6	2	2	2	66.7
Recklinghausen's disease	3	1	2		
Atrophied skin diseases					
Sclerodermia diffusa	4	2	2		
Sclerodermia en bandes	2	2			
Poikilodermia	3	3			
Hemiatrophia facialis	1		1		
Dermatochalasis	1	1			
Peripheral vascular diseases					
Acrocyanosis	9	5	4		44.4
Pernio	14	13		1	7.1
Erythromeralgia	2			2	
Raynaud's disease	1	1			
Livedo racemosa	1	1			
Osmidrosis axillae	4	4			
Hyperidrosis	4	3		1	
Hemangioma	4	3	1		
Teleangiectasia faciei hereditaria	6	6			

Infectious diseases					
Pyodermia	32	25	3	4	21.9
Tuberculosis cutis	11	8	3		27.3
Syphilis II latens	4	2	1	1	
Virulosis	9	6	2	1	
Fungus infection	4	3	1		
Scabies	3	3			
Other skin diseases	9	7	2		
Urologic diseases					
Urologic tuberculosis	8	3	4	1	62.5
Prostate hypertrophia	4		2	2	
Other diseases	13	7	3	3	
Healthy Subject	15	13	2		13.3

results of this experiments also showed a high percentage of abnormal case, the tendency being particularly marked among the patients with extensive or advanced lesions. But if the results are classified by the kinds of the diseases, except in a few items, the percentage of abnormal subjects ranged between 30% and 50%. Abnormal values were obtained in especially high frequency among eczema chronicum and erythematodes. The much higher percentage of abnormal subjects found among chronic than among acute eczema cases (including contact dermatitis) reminds us of the opinion published by Seyle that continuous or repeated irritation causes anomalies in the function of adrenal cortex and also suggests that the pituitary-adrenocortical system is capable of playing some role as a latent factor in the pathogenesis of chronic eczema. Among the erythematodes patients, the percentage is high, both in acute and in chronic cases, and in acute erythematodes, the high percentage of 62.5% was admitted. As to the pathogenesis of this disease, some researchers recently have proposed the theory of collagen disease, asserting that, in the relation of the pathogenic mechanism to the pituitary-adrenocortical system, if a disturbance of the adrenocortical hormone in the body, that is, a relative excess of mineralocorticoid over glucocorticoid hormone; persists for a long while, a degeneration of collagen fibres is caused (Selye). Many causes capable of occasioning unbalance of hormones can be thought in this case, for example, a primary undersecretion of the glucocorticoid by itself, which naturally would lead to abnormal response of the eosinophils that are under the control of the glucocorticoid. Gold[6] has suggested that the pituitary-adrenocortical system acts as a barrier against an outbreak of the disease, so that as long as the barrier is kept comaparatively sound, the disease is kept in a chronic state, but once a breach is made in this barrier, the disease breaks out in an acute form. The results of present experiments seemed to give support to this assumption to some extent. In the cases of the other allergic diseases also, abnormal values were ob-

tained in most cases where the pathological changes were advanced in degree or extensiveness, and we must wait for further investigation to answer the question whether the severity is the cause or the effect of the abnormal eosinopenia.

2) Pigmentary diseases. Many of acquired pigmentary diseases including Addison's disease showed abnormal values. The problem of the possible correlation between hyperpigmentation of the skin and the function of the adrenal cortex has been for long under debate. Recently, we have heard of the anti-SH theory by Lerner *et al.*[7], the intermedin theory by Waldenström[8] and Högberg and the melanocyte stimulaing hormone theory by Lerner and Fitzpatrik.[9] Gros *et al.*[10] have reported that following continued administration of ACTH to a patient of hemolytic anemia, an Addison's disease-like pigmentation was brought about. The author has had also recent experiences of three cases in whom, upon general improvement of Addison's disease following long-continued administration of cortisone, the pigmentation of the skin and the mucous membrane gradually subsided as Waldenström and others have observed. On the other hand, Steinberg *et al.*[11] have reported of a female patient 50 years of age, of pituitary Addison's disease. Miyazawa[12] examined of a male patient of 46 years who had skin pigmentation of Addison's disease type following basal fracture of the skull and observed that both ACTH- and epinephrine tests showed abnormal values. From these, we cannot give primary adrenocortical deficiency advocated by Waldenström, Lerner and some others. We would rather suppose that the deficiency of adrenocortical hormone itself is of significance. Among the cases of Riehl's melanosis, the cases with abnormal value were next in number to those with Addison's disease, in agreement with the results of tests reported by many investigators in Japan. Spira[13] has observed the decrease in excretion of urinary 17 ketosteroid, hypotonia and hypoglycemia in a case of this disease and discussed on its relation to adrenocortical insufficiency, while Iijima[14] reports on a case who had hypotonia and pigmentation on the buccal membrane and showed good results on prolonged administration of adrenocortical hormone. The author has also experienced of a female patient of 43 years making transition from this to Addison's disease and finally dying of it, and the author interprets that the adrenocortical insufficiency is one of the hidden internal factors leading to the outbreak of this disease, provocated by accumulation of external factors. The author may presume from the results of present experiments that in pigmentatio postlesionnelle a pathogenic mechanism similar to that of Riehl's melnosis comes into play. In the cases of chloasma, of which the relation to internal secretion, especially that from the genadic glands, is deemed to be of importance, a rate of abnormal subjects was found in far smaller than among the acquired

pigmentary diseases, and it was thought improbable that the pituitary-adrenocortical system plays any primary role in the pathogensis of this disease. In vitiligo cases, the abnormal rate was high and from the fact that nearly 20% of Addison's disease cases suffer from vitiligo as complication, the correlation between the predisposition in some of vitiligo patients and pituitary-adrenocortical function is supposed. In congenital pigmentary disease cases, the rate of subjects with abnormal values of epinephrine test was as low as normal subjects, and here we can well understand that the diseases are unrelated with the pituitary adrenocortical system, since these diseases are localized malformation in nature. However, it calls attention that nevus fuscocaeruleus ophthalmomaxillaris Ota showed higher abnormal value than nevus pigmentosus. And it is of interest that two cases of ephelides inversa showed both highly abnormal values. The coincidence of the limited positions in which these pigmentary maculae appear—the lips, the buccal mucous membrane, the palms, and the soles—with the positions where pigmentation occurs in Addison's disease seems to give a suggestion on the pathogenesis of this disease.

3) Seborrheic diseases. In acne vulgaris relative high percentage is noticed. Murayama[15] has measured the urinary 17 ketosteroid content of 38 cases of these diseases and found a relative increase of it and Kitamura *et al.*[16] tried implantation of cow's pituitary gland to such patients and admitted no case of its ellicacy, and even some cases of aggravation of the disease. Considering that these diseases occur as side-effect of ACTH administration and in cases of Cushing's disease, these diseases seem to have complicated relation with pituitary-adrenocortical system.

4) Keratotic diseases. Among cases of this group of diseases, high percentage of abnormal cases was general. The relation between dyskeratosis and internal secretion is as yet conclusively clarified, and the results of administration of various hormones have not been uniform. In psoriasis cases, Abe and Yoshimatsu have reported of 2 cases each of abnormal results among 3 cases each. Reiss, Caccalanza and others have proved decrease of urinary 17 ketosteroid in every case and Grüneberg[17] has cured with ACTH and cortisone. Present results also showed high percentage of abnormal cases. Cases of erythrodermia exfoliativa generalisata showed the highest abnormality percentage and Lever[18] has classified this disease as one of the diseases most amenable to ACTH-cortisone therapy, and these findings may be collated with the demonstration by Ohashi[19] and Sasada[20] of atrophied hypophysis and adrenal cortex in autopsy.

5) Bullous diseases. One case each of pemphigus vulgaris and foliaceus showed abnormal value. Recently, Sano *et al.*[21] have collected

deta about changes in endocrine glands from Japanese reports on autopsy of cases of these diseases, and found that in 8 cases among 10, both the hypophysis and the adrenal cortex were atrophied and degenerated. Considering this and the fact that these diseases belong to the Lever's Group 1, we may infer their significance in the pathogenic mechanism of the diseases. The results obtained in dermatitis herpetiformis may be deemed to show a similar significance.

6) Endocrine diseases. Many reports are being published on the correlation of the thyroid gland and the pituitary-adrenocortical system, but the conclusions drawn are not always uniform. In present study, the Basedow's disease cases showed normal response. Grynkewich[22] has made epinephrine tests in two cases of localized myxedema and found normal response in both cases, but one case of this disease showed abnormal value in present study. The pathogenesis of mongolism is not yet explained in a universally accepted theory. My one case of mongolism showed abnormal response. In the cases of other diseases of this group, results were obtained in general agreement with what may be expected from the accepted theories on their pathogenesis.

7) Hair diseases. Tsukada[23] studied the role of pituitary hormone on the patnogenesis of alopecia areata. Walker and Rothmann,[24] basing on clinical observations, have suggested that endocrine factors play important parts in the pathogenesis and progress of alopecia areata. Dillaha and Rothman[25] have noticed improvement by administration of cortisone to 3 cases of this disease. Wainger *et al.*[26] have reported on cases that were alleviated during administration of ACTH and cortisone but were aggravated again upon interruption of the administration. Recently, many reports of improvements following implantation of cow's pituitary gland have appeared, suggesting the close relation between this disease and internal secretion, in particular, pituitary and adrenocortical hormones. In present study, abnormal results have been obtained in 60 among 102 cases. Some cases of hypotrichosis and other diseases affecting hair also showed abnormal values.

8) Skin tumors. Not many cases with carcinoma and reticulosarcoma limited to the skin showed abnormal values, but among the cases with such tumors extended over the whole body the percentage of abnormal results was unexpectedly high. According to author's experience the adrenal cortex was found atrophied and the pituitary gland reduced in size upon autopsy of a case of Hodgkin's disease, and in one case which showed a abnormal value in epinephrine test of the two cases of mycosis fungoides,[27] infiltration of the adrenal cortex by tumor cells was proved upon autopsy. Acanthosis nigricans is explained in pathogenesis that it is caused by the obstruction of the adrenocortical function due

to the pressure caused by the carcinomatous metastasis of the coeliac plexus, by Covilland, Burmeister, Spietschka, Plollitzer, Janovsky, Sutejeff and Wainstein, Jacobsohn and others. A case of this disease showed abnormal value of epinephrine test in present study. These diseases probably are due to the hypofunction of the adrenal cortex caused by the direct invasion of the tumor tissue. It was of interest that in keloid cases showed a high percentage of abnormal results. This is in consonance with the the report by Naito *et al.*[28] that they have succeeded in suppressing keloid formation by administration of cortisone, and the observation by Plotz *et al.*[29] that healing of external wounds in repressed in rabbits and erythematodes patients while cortisone and ACTH injections are kept up, and from these findings it seems probable that a latent deficiency of adrenocortical function is present in keloidal constitution. Among the 3 cases of Recklinghausen's disease encountered, 2 cases showed questionably abnormal value, a results in consonance with the presence of adrenal and other endocrine disorders sometimes observed in patients of this disease.

9) Atrophic diseases. Sclerodermia diffusa and poikilodermia atrophicans vascularis are at present studied under the one comception of the so-called collagen diseases. Kano[30] has found abnormal results of epinephrine tests in all the 10 cases of the latter disease and was led to exphasize a deficiency of the pituitary-adrenocortical system as its cause, but my tests showed normal results in all cases. If we admit Seyle's theory that the pathogenic mechanism lies in a dysharmony of mineralocorticoid and glucocorticoid hormones, it is supposed that the presence of dysfunction in the pituitary-adrenocortical system is not always a necessary condition.

10) Peripheral vascular diseases. 2 cases of erythromeralgia following mushroom poisoning showed highly abnormal values, but these values were obtained in the gravest stage of the disease. About half of the acrocyanosis cases showed abnormal results, but the patients of pernio and Raynaud's disease showed normal values in all cases.

11) Infectious diseases. The unrelatedness with the endocrine system had been anticipated, and in fact only a few cases with extremely advanced or widely extended inflammation showed abnormal values.

12) Urogenital diseases. Among the urogenital tuberculosis cases more than half of the total showed abnormal results, but seeing that almost all of our tested cases were patients with tuberculosis of both kidneys, we may consider the probable dysfunction of the adrenal glands as due to the invasion of tuberculosis inflammation or its pressure in these cases. The cases with prostate hypertrophy showed abnormal results in every case, and it is supposed that there are any senile hormonal dishormony in the pathogenesis of this disease.

Conslusion

The author has subjected 955 cases with various skin diseases to epinephrine test and has obtained the following results.

1. The allergic disease in general showed high percentages of abnormal cases, especially when the lesions were wide-spread or highly advanced, the tendency was accentuated. Many cases showed results interpretable as Thorn's so-called false response. The acute erythematodes showed unusually high percentage of abnormal cases, reflecting the probable participation of a deficiency in the pituitary-adrenocortical system in the pathogenesis of these diseases.

2. Among pigmentary diseases, the cases of acquired pigmentry diseases showed a high percentage of abnormal results, but the congenital pigmentary disease cases excepting ephelides inversa had values not different from those of normal subjects.

3. The seborrheic diseases generally showed a low percentage of abnormal cases. On the other hand, many of the keratotic, bullous and hair disease cases, especially alopecia areata cases, showed abnormal values. The existence of an endocrine background in the pathogenesis of these diseases is presumed.

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On Epinephrine Tests in Skin Diseases. II

By

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I. Comparison of Results of ACTH- and Epinephrine Test

A comparison of the results of the two kinds of test effected on 37 cases at intervals of 2~3 days showed the following results:

Results −50% and above (+), and −49% or less (−)

a) 22 cases of uniform results (59.5%)
   (−) in both tests 5
   (+) in both tests 17

b) 15 cases of discrepant results (40.5%)
   (+) in ACTH test and (−) in epinephrine test..... 2
   (−) in ACTH test and (+) in epinephrine test..... 13

Lohmeyer and Hüsselman[1] made both tests in 32 cases and obtained uniform results in 17 and discrepant results in 15, and were led to express very skeptic views on the clinical value of epinephrine tests. In Japan also, Ohmori[2] and Abe observed similar results and has given their doubts on the reliability of epinephrine tests. The author's results seem to agree with the results obtained by Lohmyer *et al.* at a glance, but upon more detailed examinations they can bring opposite conclusions. That is: 1) The rate of average decrease of the eosinophils in 37 cases amounted to −32% upon ACTH tests and to −40% following epinephrine tests, the latter being more marked. 2) The percentage of decrease upon epinephrine tests were below those upon ACTH tests in 8 cases only, in all the other 29 cases the epinephrine tests giving always higher ratio of decrease than that of ACTH. 3) Among the cases of discrepant results, only 2 cases showed (−) upon epinephrine test and (+) upon ACTH tests but in the other 13 cases showed normal values upon epinephrine testw but (+) upon ACTH tests. This, if the percentage of decrease of eosinophils alone is considered, epinephrine eosinopenia is more apparent than ACTH eosinopenia, it is generally safe to assume that cases showing (−) results upon epinephrine tests will give negative results upon ACTH tests too. From the above, it is

concluded that though, may be, epinephrine tests cannot entirely supersede ACTH tests, they are at least useful to some extent.

II. Relationship between Epinephrine Test Results and Clinical Process

The results of tests repeated 2~8 times each on 79 skin disease cases within a period of observation of 7 days to one year were as summarized in Table I.

TABLE I

Epinephrine test results (Compaired with initical value)	Clinical process	Cases
Improve (decrease more than 20%)	Improve	24
	Unchange	4
	Aggravate	0
Unchange (change within 20%)	Improve	13
	Unchange	27
	Aggravate	0
Aggravate (increase more than 20%)	Improve	4
	Unchange	4
	Aggravate	1

As to the relation of the progress of disease and the epinephrine test result, Ohmori *et al.*[2] have observed agreement in 45% and in present study in 77 cases, parallel results were obtained in 52 cases (67.5%). So it may be said that epinephrine tests show the state of progress with considerable accuracy. We conducted implantation of cow's pituitary aglnds one to seven times each in alopesia areata in 24 cases, but little constancy was found in the relation between the progress of their diseases and the results of epinephrine tests, except in 3 cases who showed improved test results accompanying clinical improvements.

III. Variations of Epinerphrine Test Results during continued Administration of Cortisone

Two cases of Addison's disease and one each of pemphigus vulgaris, erythema nodosum and mongolism cases were subjected to epinephrine tests before, during and after cortisone treatment and the changes in the results followed up.

Case No. 1. Male, 29 years, Additon's disease. The test result was −24% before the initiation of cortisone treatment, −40% on the 20th day of the treatment when the administered cortisone totaled 675 mg., and on the 57th day, when the total dosis reached 1,145 mg., became

−61%.

Case No. 2. Male, 40 years, Addison's disease. Test result before treatment, −39%; on 89th day (total cortisone dosis, 1,370 mg.), −51%.

Case No. 3. Male, 61 years, pemphigus vulgaris. Test result before treatment −44%; on 33rd day (total cortisone dosis, 3,300 mg.), −56%; 34th day after discontinuance of treatment, −33%. This patient had a relapse of eruption soon after discontinuance of the treatment and though the eruption healed upon readministration of cortisone treatment, died of general marasm. Upon autopsy, frank atrophia and degeneration were apparent in the pituitary and adrenal cortex.

Case No. 4. Female, 30 years, erythema nodosum. Test result before treatment, 13%; on the 10th day (total dosis of cortisone, 1,000 mg.), −57%; 34th day after discontinuance of treatment, −33%.

Case No. 5. Female, 30 years, mongolism. Test result before treatment, 15%; on the 24th day of treatment (total dosis of cortisone, 1,370 mg.), −55%; 15th day after discontinuance of treatment, −36%.

Kark *et al.*[3] and Waldenström[4] have found normal values as epinephrine test results of surgical Addison's disease who had their adrenal glands on both sides excised and had been treated with cortisone on account of prostate carcinoma, and expressed their doubts on the clinical value of this test, but in author's cases, it was admitted that the test vslues normalize gradually during cortisone treatment and in Case No. 3 and 4, a relapse to the pretreatment values upon suspensino of the treatment. These improved results during cortisone-treatment apparently does not mean sheer improvement in the function of the pituitary-adrenaocorticl system, but we must take transitional effects of the administered cortisone into consideration. The mechanism leading to eosinophenia following epinephrine tests is not yet adequately explained, but a solution might be sought in the hypothesis by Walderström that epinephrine causes eosinopenia by release of the corticoid depots in the body, Now, since a continued administration of cortisone involved an accumulation in the body to some extent, the epinephrine in such cases, beside directly influencing the pituitary-adrenocortical system, may be supposed to effect a release of the depots of accumulated cortisone and thus enhance the resulting eosinophenia.

IV. Behavior of Circulating Eosinophils under Antigen Loading Test

The author have made the experiments described hereunder for the purpose of elucidating the influence of the allergic reaction on the pituitary-adrenocortical system in certain cases of allergic diseases with the changes of eosinophils in blood as index.

Description of cases and experimental method: One case each of dietetic urticaria and urticaria perstans papulosa and 2 cases each of drug exanthema and cold urticaria were subjected to epinephrine and antigen

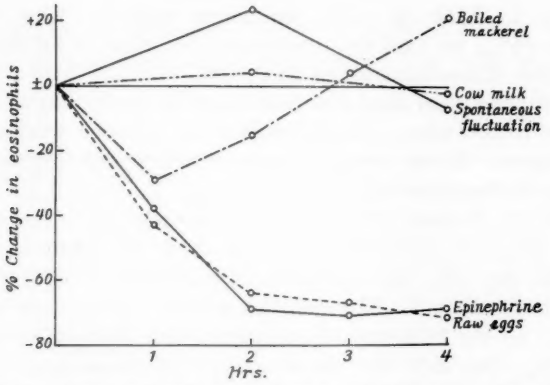


Fig. 1. Dietetic urticaria, male, 19 years. 20 minutes after eating 2 raw eggs, itching and general urticaria developed, but upon feeding 300 cc. of milk or 50 g. of boiled mackerel, no anomaly occurred.

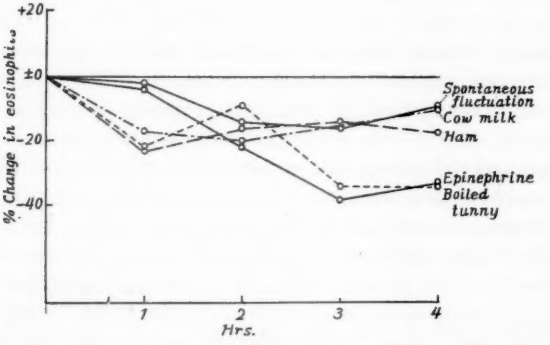


Fig. 2. Urticaria perstans papulosa, male, 33 years. By taking 50 g. of boiled tunny, itching and reddening came out at the eruptions. No reaction was observed upon taking in milk or ham.

loading tests at 1～2 days intervals. Latter tests includes orally given one full meal or one dosis of the drug. The eosinophils were counted before and every one hour after the antigen administration.

Results. As shown in Figs. 1～6.

In all the 6 cases above, the decrease of circulating eosinophils showed the tendency of being emphasized in paralled with the intensity of the

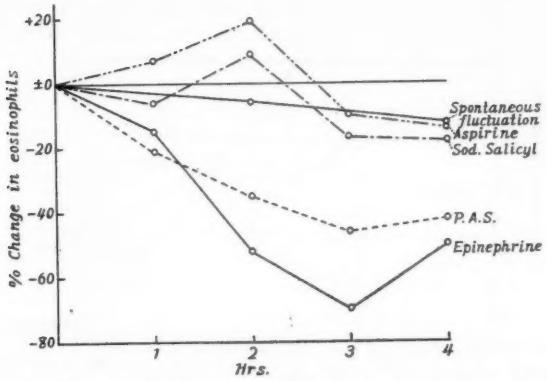


Fig. 3. Drugexanthema (PAS), male, 40 years. 30 min. after administration of 2 g. of PAS, exanthemes, developed all over the body and vomiting followed. No reaction observed upon administration of sodium salicylicum, aspirin etc.

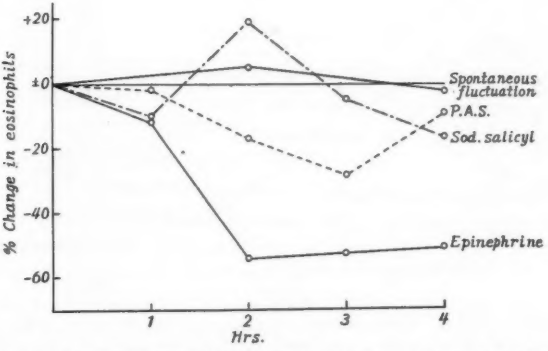
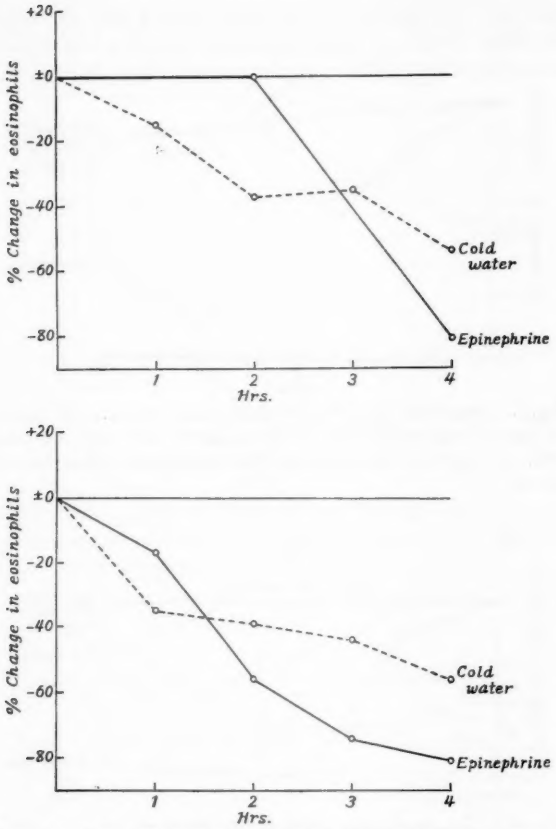


Fig. 4. Drugexanthema (PAS), male, 38 years. Upon administration of 2 g. of PAS, slight itching was felt, but no exanthema was noted.

reaction i.e. the degree of eruption during antigen loading tests. Besides, when the curves of the circulating eosinophil counts as the time of epinephrine administration, at the time of antigen loading, at the time of administering non-antigen substances and the spontaneous fluctuation curve under no loading are compared, the author find generally a parallel lowering of the curves at the time of antigen loading and at the time of epinephrine administration. Also a general resemblance of the curve at the time of non-antigen substance administration and to that of spontaneous fluctuation under no loading is noticed. If this result is to be explained by the so-called general adaptation syndrom theory of Selye, it may be said to reflect the



Figs. 5 and 6. Cold urticaria, females, 15 and 18 years. One of the feet was immersed in cold water (11°~13°C) for 30 min. each. Soon a frank urticaria developed, extending from the lower to the upper things.

requirement of strong endocrine adaptation against the antigen antibody reaction caused in the body by administration to subject with allergic disposition of certain drugs or food that would have no effect upon subjects with no allergic disposition. In this case, the reaction mechanism would be interpreted, as Selye says, as consisting in a chain reaction in the pituitary-adrenocortical system following of histamin or histamin-like substances produced by the allergic reaction with the antigen as stressor.

V. Relation of Epinephrine Test to Urinary Content of 17 Ketosteroid

The results of epinephrine tests and measurements of urinary 17 keto-

steroid content by Holtroff and Koch's method conducted on 25 skin disease cases were studied in comparison.

Results. As thown in Table II.

TABLE II

Urinary excretion of 17 K.S. \ Epinephrine test results	Normal	Abnormal
Normal	4	4
Decrease	5	12

Many investigators have conducted epinephrine tests and 17 ketosteroid determinations in combination, but their results have not been constant, so that Posey *et al.*[5] have declared the two as unrelated, but Miyake, Baba and others have seen a generally parallel relation between the two. In present study, the author have found in 16 cases (66.7%) among the total 25 cases a parallelism of the results.

Conclusion

1. The results of ACTH and epinephrine tests showed agreement in 60% of the tested cases. The latter test in superior to the former, and the subjects thowing negative results in epinephrine tests may be without much error to give negative results in ACTH tests also.
2. The relation between the clinical progress and the results of epinephrine tests showed parallelism in about 70% of the testsed cases. Thus epinephrine test may be presumed to indicate the state of illness with considerable accuracy.
3. Continued cortisone administration normalized the results of epinephrine tests in the majority of the cases.
4. The author succeeded in demonstrating that an internal introduction of antigen in allergic skin disease cases calls for an intense endocrine adaptation from the standpoint of the theory of general adaptation syndrome.
5. The results of epinephrine tests and measurement of the urinary content of 17 ketosteroid showed parallel changes in 70% of the tested cases.

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Plasma Prothrombin Time in Skin Diseases

I. Whole and 12.5% Plasma Prothrombin Time in Skin Diseases

By

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Blood prothrombin had been determined on whole plasma until Campbell *et al.*[1] employed it on plasma diluted with saline to the concentration range 25 to 5%. Shapiro[2] noted the importance of the difference between prothrombin times of whole and 12.5% diluted plasma which is fairly constant in normal individuals and said that when the difference is small it may signify hyperprothrombinemia or an excess of anticoagulants in tne blood. Brambell and Loker[3] also stress that plasma prothrombin time for diluted plasma is a more sensitive indicator of prothrombin activity than is the whole plasma. Abe[4] recently said that prothrombin is a complex of components A and B (not identical to those of Quick) and concluded that 12.5% plasma prothrombin time depends chiefly upon component B which is rather stable by preservation than component A.

The present study is based on the one-stage whole and 12.5% saline diluted human plasma prothrombin time (P.P.T.) in various skin diseases. Materials are the patients who visited or were hospitalized in our department during the period from 1950 to 1954.

Materials. The method is fundamentally similar to that of Matsuoka[5] but the author modified some points as follows: 1) Thromboplastin. Rabbit weighing about 3 kg. is killed by air embolism. Cut the scalp aboiding minimal damage. Put the brain with whole meninges in distilled water. Take off the meninges and small bloodvessels carefully. The brain in placed in a mortor and macerate 30 minutes. Cooled redistilled aceton (B. P. 56°C) is poured over pap and stirred 5 min. Then whole contents of the mortor is centrifuged at 3000 r.p.m. for 15 min. Remove supernatant fluid with palpy substance at the top of centrifuge tube. Add ca. 20 cc. of cooled, redistilled aceton, stir and centrifuge at 3000 r.p.m. for 15 min. After four times aceton dehydration and agitation, the de-

posit is placed on a sheet of filter paper, dried at 37°C for 10 hours. About 0.3 g. of dried brain powder, pale ochre in color, is obtained. Kept in $CaCl_2$ desiccator at 4°C. 2) Thromboplastin Suspension. Put 0.3 g. of dried brain in a test tube containing 5 cc. of 0.85% fresh saline solution. Test tube is immersed in a 45°C water bath which is heated by two electric lamps one of which is regulated by toluenmercury thermostat. In the test tube a glass rod with spatula-like tip which rotates by the string of the pulley of stirler of the water bath is immersed. The agitation is ended after 15 min. Cool the contents and after 3000 r.p.m. 15 min. centrifuging, supernatant fluid is used. 3) 1/10 *N* $CaCl_2$ aqueous solution. As the reagent is absorbable, approximate aqueous solution is corrected by $AgNO_3$ titration. 4) 1.4 g./d*l*. potassium oxalate aqueous solution. 5) Human plasma. 1.8 cc. of human blood is withdrawn in a 2 cc. tuberculine syringe which contains 0.2 cc. of 1.4% potassium oxalate solution. After 3000 r.p.m., 10 min. centrifuging, supernatant not hemolized plasma is used. 12.5% plasma: 0.1 cc. of patient's plasma was diluted with 0.7 cc. of 0.85% saline.

Technic. As the patients tested were out-patients, the venipuncture was done after breakfast. In a 16 mm. × 100 mm. test tube put 0.1 cc. each of patient plasma and thromboplastin suspension in this order. Immerse the test tube in the water bath (37°C±1°C) for 6 min. then add 0.1 cc. of $CaCl_2$ solution warmed at 37°C and start a stop watch. After slow rotation of test tube by fingers in the bath (once in 2 seconds,) gelatineous coagulation is noticed and the contents of the test tube cease to flow freely. Stop the watch at that moment and note the time. The coagulation phase is different in whole and diluted plasma i.e. coagulation is momentary in the former and time-interval is required from the onset to end-point in the latter and the author takes the endpoint.

Results. Meanvalue of each duplicate determination of the whole and 12.5% P.P.T. are shown in Table I. Physiological range is 13.0±1 seconds in whole and 40.0±10 seconds in 12.5% plasma as shown at the bottom of Table I.

Comment

Allergic diseases. In contact dermatitis protracted P.P.T. is found in repeated cases, wide-spread cases and cases which have pronounced edema and extensive secretion even the site is localized merely on face or on extermities as in the results of Takata reaction by Iwama.[5] In most of the cases within physiologic range, the lesion presents papula and scale on circumscribed erythma. In eczema acutum most of erythematosquamosum type is found in normal range. Protraction is evident in exsudative stage like contact dermatitis. The difference between these two

TABLE I

Diagnosis	Cases	Range of whole and 12.5% plasma prothrombin time in seconds ( ) shows 12.5% P.P.T.						Percentage of protracted cases	
		22.1 and above (130.1 and above)	20.1~22.0 (110.1~130.0)	18.1~20.0 (90.1~110.0)	16.1~18.0 (70.1~90.0)	14.1~16.0 (50.1~70.0)	11.0~14.0 (30.0~50.0)	Whole P.P.T.	12.5% P.P.T.
Allergic Diseases									
Contactdermatitis	58			5 (5)	15 (10)	16 (26)	22 (13)	26.0	74.0
Eczema acutum	29				2 (2)	15 (11)	12 (14)	59.3	48.1
Eczema chronicum	29		1	2 (1)	3 (4)	10 (22)	13 (1)	55.1	96.5
Neurodermatitis	24				5 (1)	6 (5)	4 (14)	45.8	30.0
Prurigo Besnier	11			1 (1)	2	8 (4)	(4)	100.0	60.0
Prurigo Hebra	5					2 (2)	3 (4)	40.0	33.3
Prurigo nodularis	3					3 (2)		100.0	100.0
Pruritus cutaneus	9			(1)	1 (1)	5 (2)	3 (4)	66.6	50.0
Urticaria	26			2 (1)	10 (6)	7 (8)	7 (1)	73.0	60.0
Quincke's edema	1		(1)	1					
Erythema exsudativum	26			2 (3)	8 (3)	7 (7)	7 (7)	70.4	65.0
Erythematodes	21		3	(2)	8 (7)	8 (8)	2	90.5	100.0
Poikilodermia	2				2 (2)				
Weber-Christian's disease	1			1					
Erythema nodosum	9			1	3 (1)	3 (4)	2 (4)	77.7	55.5
Purpura	22			(2)	2 (6)	14 (8)	6 (5)	72.7	76.1
Toxic eruption (dietary)	22			4 (3)	6 (4)	4 (8)	8 (7)	63.6	68.1
Drug exanthema	19		2	6 (2)	2 (4)	4 (8)	5 (5)	73.6	73.6
Ectodermose erosive	1			1	(1)				
Sebrrhoic Diseases									
Eczema seborrhoicum	28			(1)	4 (4)	14 (15)	10 (7)	64.2	74.0
Acne vulgaris	12					2	10 (12)	20.0	
Acne rosacea	5						5 (5)		
Keratotic Disease									
Psoriasis	19		2	1 (1)	5 (5)	4 (6)	7 (4)	63.1	75.0
Pityriasis rubra pilaris	5				(1)	2 (1)	3 (2)	40.0	50.0
Lichen ruber	4				1	2 (3)	1 (1)	75.0	75.0
Erythrodermia generalisata	8			1	1 (1)	2 (2)	4 (1)	50.0	75.0
Pityriasis rubra Hebra	6			2	2 (1)	2		100.0	100.0
Keratodermia tylodes palmaris progrossiva	12				1	2 (3)	9 (9)	25.0	25.0
Bullous Disease									
Herpes zoster	8		1	(1)		1 (1)	6 (6)	25.0	25.0
Dermatitis herpetiformis	5				1 (2)	3 (2)	(1)	100.0	80.0
Pemphigus vulgaris	2			1	(1)	(1)	1	50.0	100.0
Bullosis mechanica	1					(1)			
Hydroa vacciniformis	1				1 (1)				
Pigmentary Diseases									
Vitiligo	17					2 (2)	15 (15)	11.7	11.7
Acquired leucoderma	8					(1)	8 (7)	0	12.5
Acquired pigmentation	5						5 (5)		
Nevus pigmentosus	14						14 (14)		
Ota's nevus	7						7 (7)		
Ephelides	4					1 (1)	3 (3)	25.0	25.0
Xeroderma pigmentosum	1						1 (1)		
Dyschromatosis univ.	1					(1)	1		

TABLE I—Concluded

Diagnosis	Cases	Range of whole and 12.5% plasma prothrombin time in seconds ( ) shows 12.5% P.P.T.						Percentage of protracted cases	
		22.1 and above (130.1 and above)	20.1~22.0 (110.1~130.0)	18.1~20.0 (90.1~110.0)	16.1~18.0 (70.1~90.0)	14.1~16.0 (50.1~70.0)	11.0~14.0 (30.0~50.0)	Whole P.P.T.	12.5% P.P.T.
Chloasma	8					2 (3)	6 (5)	25.0	37.5
Riehl's melanosis	6				1	2 (5)	3 (1)	50.0	83.4
Addison's disease	9		(1)	1	2 (1)	4 (5)	2 (2)	77.8	77.8
Perniosis	16			(1)	1	7 (7)	8 (8)	50.0	50.0
Burns (extensive)	3	(1)	1	1 (2)	1			100.0	100.0
Congenital Diseases									
Ichthyosis	7				(1)	3 (2)	4 (3)	42.6	42.6
Lichen pilaris	2					2 (2)		100.0	100.0
Keratosis palm. plant. hered.	4				1	2 (2)	1 (2)	75.0	50.0
Darier's dyskeratosis	1					(1)	1		
Keratosis follicularis squamosa Dohi	1						1 (1)		
Teleangiectasia faciei	6					(1)	6 (5)		16.6
Osler's disease	1					1 (1)			
Recklinghausen's disease	3					1 (2)	2 (1)	33.3	66.6
Unna's angioma	1						1 (1)		
Mongolism	1				1	(1)			
Tuberculosis									
Cutaneous tuberculosis	7			1	1	2 (5)	3 (1)	57.1	83.3
Tuberculides	14			1 (1)	4 (1)	2 (5)	7 (4)	35.2	71.4
(Urogenital tbc.)	14					10 (6)	4 (8)	71.4	42.8
Pyodermia	10			1 (3)	4 (2)	1 (2)	4 (3)	60.0	70.0
Fungus infection	16			1	1 (1)	6 (10)	8 (5)	50.0	50.0
Lepra tuberosa	1				1 (1)				
Tumor									
Keloid	2					(1)	2 (1)		50.0
Paget's disease	2					2 (1)	(1)	100.0	50.0
Bowen's disease	1						1 (1)		
Ulcus rodens	4				1 (1)	2 (1)	1 (2)	74.9	50.0
Reticulosarcoma cutis	1					1 (1)			
Hodgkin's disease	2			1	(1)	1 (1)			
Acanthosis nigricans	1					1 (1)			
Endocrine Diseases									
Acromegalia	1				(1)		1		
Diabetes incipidus	1				1	(1)			
Pituitary infantilism	1					1	(1)		
Other Diseases									
Hemiatrophia	2					1	1 (2)	50.0	
Sclerodermia	3				1 (1)	1 (2)	1	33.3	100.0
Striae distensae	1						1 (1)		
Alopecia areata	3					1 (1)	2 (2)	33.3	33.3
Ariboflavinosis	3				(1)	2 (1)	1 (1)	66.6	66.6
Livedo reticularis	2			1	1 (1)	(1)		100.0	100.0
Acrocyanosis	3					2 (2)	1 (1)	66.6	66.6
Vascular spider	1				1	(1)			
(Liver Diseases†	11	2 (4)	(2)	5 (3)	2	2 (4)		100.0	100.0)
(Urogenital Diseases*	16				(1)	5 (5)	10 (7)	37.5	46.1)
Healthy	30					3 (3)	27 (25)		

† Liver diseases are examined by comparison, cases are 4 hepatitis acuta, 3 cirrhosis, 2 serum hepatitis, one case each of liver syphilis and choledochus carcinoma. Both P.P.T. is absolutely protracted in all cases. * Urogenital diseases contains gonorrhoea, strictura urethrae, nephrolithiasis, ren mobilis, varicocele, and enuresis. 5 cases of hypertrophia prostatica present no difinite protraction in both P.P.T.

diseases is the higher protraction ratio of the former than that of the latter in 12.5% P.P.T. Most of eczema chronicum shows pronounced protraction of 12.5% P.P.T. and protraction of whole P.P.T. is found especially in en plaques and follicular type. In neurodermatitis no difference is found in diffuse and circumscribed types. Prurigo Besnier and nodularis give higher protraction than neurodermatitis. In urticaria protraction is distinct in acute manifest forms rather than chronic form. A case of urticaria perstans papulosa exhibits normal P.P.T. in whole plasma but slight protraction in 12.5%. Cases of exudative erythema consists from 19 exsudativum multiforme, 5 annulare centrifugum Darier and one annulare angioneuroticum. No difference of P.P.T. in these three types is found and protraction is evident in fresh cases. Erythematodes (18 discoides chronicus and 4 disseminatus acutus) shows 100% protraction of 12.5% P.P.T. in contrast with that of 90.5% in whole P.P.T. Even in the beginning of the disease protraction is evident in erythematodes. In two cases of poikilodermia and a case of Weber-Christian's disease protracted P.P.T. is noticed. In short, absolute protraction of P.P.T. is noticed in socalled collagen diseases. In purpura (8 cases each of simplex and rheumatica types, one Henoch type, 3 Majocchi types and 2 Werlhof types), each protraction rates of five types are different in whole and dilute plasma. In toxic eruption chiefly caused by foods no difference is found in exsudativum multiforme, scarlatiniforme and Gibert types but protraction seems to be found in acute cases.

In three cases of Penicillin exanthema, one case presents the high protraction, one case of erythrodermia generalisata type shows moderlate but one case with erythema localyzed on the upper arms on the face is found in normal range. PAS-exanthema (6 cases) and Sulfadrug-exanthema 3) cases) show moderate protraction of both P.P.T. Fixed-exanthema (2 cases), Tibion-exanthema (one case) and Salvarsan-exanthema (2 cases) show slight protraction.

In seborrhoic diseases acne vulgaris and rosacea are found in normal range but eczema seborrhoicum presents protraction of both P.P.T. when the disease is localized on head. According to Katabira[7] of our clinic urobilinogen in urine is positive in 15% and Takata reaction is 66% in 135 eczematide cases. In psoriasis (14 vulgaris, one arthropathica and 3 parapsoriasis guttata) the protraction is 63.1% in contrast with the positive ratio of 37.5% in Takata reaction (Iwama[6]). Pityriasis rubra Hebra presents 100% protraction in both P.P.T., but the protraction in generalized erythrodermia is not manifest, especially in the onset, but protraction is noticed in recidivance types. Eight cases of keratodermia tylodes palmaris progressiva (eczema tyloticum-like chronic disease predisposes young women in Japan) with above 3 years duration show slight

protraction in 12.5% P.P.T. A 44 years old female with herpes zoster presented marked protraction of both P.P.T.

In bullous diseases protraction is noticed in dermatitis herpetiformis, pemphigus vulgaris and hydroa vacciniformis but bullosis mechanica senilis presents slight protraction in 12.5% P.P.T.

In pigmentary diseases the protraction is noticed in Riehl's melanosis and Addison's disease. Most of vitiligo are in normal range but in two cases of Harada's disease slight protraction of both P.P.T. is noticed. In perniosis no findings of variation in P.P.T. corresponding to the type and grade of the disease is found. All 3 of combustio cases are fresh and widespread bullosa type.

According to Komori P.P.T. in tuberculous patient is protracted but Okabe[8] denied the marked hypoprothrombinemia. In author's experiment cutaneous tuberculosis presents moderate protraction of 12.5% P.P.T. in contrast with very slight protraction of whole P.P.T. In urogenital tuberculosis as a control this relation is antipodal. Case of lepra is an acute tuberosa type. In infectious diseases 12.5% P.P.T. protraction is higher than that of fungous infections.

Hodgkin's disease with severe pruritus cutaneous presents marked protraction of whole P.P.T. but in another case the protraction is slight in both P.P.T. Moderate protraction of both P.P.T. is noticed in a case of ulcus rodens pigmentosus. Endocrine diseases are normal except for protracted whole P.P.T. in a case of diabetes insipidus. In sclerodermia, diffusa type is protracted higher than en bandes types. Vascular spider complicated with acne rosacea show slight protraction of 12.5% P.P.T.

Conclusion

Plasma prothrombin time is determined on whole and 12.5% saline diluted plasma in 665 cases of skin diseases including 316 allergic diseases.

1. High protraction rates of both whole and 12.5% plasma prothrombin time are noticed in allergic diseases especially in erythematodes, poikilodermia and Weber-Christian's disease.

2. Pityriasis rubra Hebra, dermatitis herpetiformis, pemphigus vulgaris, psoriasis and eccema seborrhoicum present also protracted plasma prothrombin time but no definite protraction is found in congenital, endocrine and pigmentary diseases except for Addison's disease and Riehl's melanosis.

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